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รายงานวิจัยฉบับสมบูรณ์

โครงการการควบคุมโรคธาลัสซีเมียชนิดรุนแรงด้วยวิธีก่อนคลอด

Prenatal Control of Severe Thalassemia Syndrome

ผู้วิจัย

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และสำนักงานกองทุนสนับสนุนการวิจัย

Abstract

Thalassemia is the most common hematologic genetic disease in Thailand. The prevalence of α-thalassemia-1, β-thalassemia, and HbE gene in our population is as high as 14%, 3-9%, and 13% respectively, leading to many births of children with severe thalassemia, including homozygous β-thalassemia, β-thalassemia/HbE, and Hb Bart's disease. The affected persons of the first two entities have low quality of life, while Hb Bart's hydropic fetuses have never survived but are associated with serious obstetric complications such as pre-eclampsia, dystocia and postpartum hemorrhage. Therefore, these three entities of the disease need to be controlled, especially by prenatal approach. We have launched the strategy for prenatal control in our hospital for several years. However, though we have success with preliminary project, several problems need to be solved. We need to know what the best strategy is, what is the best screening and diagnostic test for application use in the real practice? The objectives of this project were to compare various screening tests and diagnostic tests for thalassemia carriers in term of clinical use and to determine the effectiveness of prenatal strategy in reducing new cases of severe thalassemia. Additionally, the projects have tried to establish normal reference range of various ultrasound parameters to for Thai population to help early prenatal diagnosis of the severe forms. Finally, this project studied on efficacy of various sonomarkers in identification of hemoglobin Bart's disease.

Some primary outcomes of this project may be summarized as follows:

- Based on our multicenter study on effectiveness of the strategy of prenatal control of severe thalassemia on more than 8000 pregnant women, we have found that the strategy could prenatally identify affected fetuses with a detection rate and negative predictive value of 100%. However, false positive was about 75%, consistent with theoretical risk of having affected fetuses 25%. Overall, we conclude that the strategy are very effective and are able to prenatally detect all affected fetuses with an acceptable false positive rate.
- The effectiveness of the simple screening tests for α thalassemia1 / β thalassemia1, including OFT, MCV and MCH is comparable but MCV may be the best due to its convenience and highest accuracy and may be the test of choice in widely implementation.
- A comparison of the accuracy of various HbE screening tests reveals that CMU-E screen was the best, giving a sensitivity of 100% with very low false positive rate and this test may be considered to be the test of choice in widely implementation in area of high prevalence.

- Alpha-thal-IC strip test had high sensitivity for α thalassemia1 gene. All cases with α thalassemia1 trait would give a positive result. However, it had a rather high false positive rate, more than 10%. It cannot replace PCR, but it may be an option for a secondary screening test for α thalassemia1 for further testing with PCR.
- This project studied on several aspects of sonomarkers in detecting fetuses with Hb Bart's diseases. Several fetal parameters were thoroughly evaluated both anatomical and Doppler parameters. Cardiac marker and Doppler velocity of the middle cerebral artery are the best but other parameter can be used as an adjunct.
- This project also developed sonographic reference ranges of various fetal parameters, as a basic tool in evaluating Thai fetuses, for example liver length, splenic artery peak systolic velocity, splenic circumference, ductus venous Doppler velocity, etc.
- Unfortunately, some minor projects could not be successfully done for example a study on fetal loss related to the invasive prenatal diagnosis. This could not be performed due to small sample size to gain power of test for study.

บทคัดย่อ

ธาลัสซีเมีย (thalassemia) เป็นโรคที่เกิดจากความผิดปกติทางพันธุกรรมของเม็ดเลือดแดง เป็นโรคที่พบมากในประชากรไทย อาการของโรคมีตั้งแต่โลหิตจางเล็กน้อยไปจนถึงรุนแรงมาก หรือเสียชีวิตตั้งแต่อยู่ในครรภ์ หรือช่วงสั้นๆ หลังคลอด การควบคุมธาลัสซีเมียชนิดรุนแรงด้วยยุทธวิธีก่อนคลอดจะช่วยลดจำนวนผู้ป่วยและค่าใช้จ่ายในการรักษา แนวคิดในการป้องกันและควบคุมโดยวิธีคัดกรองและวินิจฉัยก่อนคลอด ซึ่งได้รับการพิสูจน์แล้วว่ามีประสิทธิภาพสูง ประกอบด้วยหลักการสำคัญคือ 1) ให้ความรู้ โดยเน้นความเป็นพาหะ ความเป็นคู่เสี่ยง และความเสี่ยงต่อการมีลูกเป็นโรค 2) การตรวจคัดกรองหาพาหะ โดยเฉพาะอย่างยิ่งในขณะตั้งครรภ์ ซึ่งถ้าสตรีเป็นพาหะ ต้องคัดกรองสามีดูว่าเป็นพาหะด้วยหรือไม่ 3) การให้คำปรึกษาทางพันธุศาสตร์ ให้คู่สมรสเข้าใจถึงความเสี่ยง ธรรมชาติของโรค การหลีกเลี่ยงการมีลูกที่เป็นโรค 4) การวินิจฉัยก่อนคลอด (ในรายที่เป็นคู่เสี่ยง) และ 5) การให้ทางเลือกแก่คู่สมรส ในการตัดสินใจยุติการตั้งครรภ์ หรือเตรียมพร้อมกับการดูแลบุตรที่เป็นโรค แนวทางป้องกันและควบคุมโรคธาลัสซีเมียก่อนคลอดดังกล่าวจะช่วยพัฒนาคุณภาพชีวิตของคนไทยและลดค่าใช้จ่ายของประเทศได้ทางหนึ่ง ซึ่งยุทธวิธีการควบคุมมีหลายรูปแบบ ซึ่งมีความเหมาะสมแตกต่างกันไปตามยุคสมัย หรือภูมิภาคที่มีความชุกและเศรษฐานะแตกต่างกัน โครงการการควบคุมโรคธาลัสซีเมียชนิดรุนแรงด้วยวิธีก่อนคลอดได้ทำการศึกษาค้นคว้าหาในด้านต่าง ๆ ที่ได้กล่าวมาข้างต้น ซึ่งได้ผลดังต่อไปนี้

- เปรียบเทียบความถูกต้องในการคัดกรองพาหะ α thalassemia1 / β thalassemia1 ของ OFT, MCV และ MCH พบว่าผลใกล้เคียงกัน แต่ MCV จะให้ผลเหนือกว่าเล็กน้อย และมีความสะดวกจึงได้เลือกให้เป็นเทคนิคลำดับแรกของการคัดกรอง alpha-/beta thalassemia
- เปรียบเทียบความถูกต้องในการคัดกรองพาหะ Hb E พบว่า E-Screen (CMU-E screen) Test ดีที่สุด มีความไวถึงร้อยละ 100 และผลบวกลวงต่ำมาก จึงแนะนำให้เป็นเทคนิคลำดับแรกของการคัดกรอง HbE
- ความถูกต้องในการวินิจฉัยพาหะ α thalassemia1 ของ alpha-thal-IC strip มีความไวสูงมาก แต่มีผลบวกลวง อยู่มากกว่าร้อยละ 10 แม้จะเป็นการทดสอบที่สะดวก ใช้ได้แพร่หลาย ไม่จำเป็นต้องมีเครื่องมือการตรวจราคาแพงแบบ PCR แต่ก็ยังไม่สามารถนำมาใช้ทดแทน PCR ได้ แต่อาจใช้ IC-strip เป็น option หรือ secondary screening เพื่อคัดเลือกบางรายไปตรวจ PCR หรือในรายที่ให้ผลบวกให้ตรวจอัลตราซาวด์แทนการตรวจ PCR (ไม่ต้องเจาะเลือดสายสะดือ)
- การศึกษาประสิทธิภาพของยุทธวิธีก่อนคลอดในการวินิจฉัยธาลัสซีเมียก่อนคลอด ซึ่งเป็นการศึกษาหลายสถาบัน (multicenter) หลังจากที่ได้พยายามศึกษาวิธีการคัดกรองใน project 1-3 แล้ว ได้นำวิธีการที่คัดกรองและวินิจฉัยก่อนคลอดมาประยุกต์และประเมินผล ซึ่งนับว่าได้ผลดี ดังที่ได้แสดงใน project 4 (ผลงานตีพิมพ์ชิ้นที่ 20)
- การศึกษาถึงภาวะแทรกซ้อนต่าง ๆ ของหัตถการเพื่อการวินิจฉัยก่อนคลอด ไม่สามารถทำได้ตามที่ตั้งใจไว้ตั้งแต่ต้น เนื่องจากการศึกษาในหลายสถาบัน หลีกเลี่ยงการเจาะเลือดสายสะดือ หรือ CVS ไปเป็นการเจาะน้ำคร่ำ หรือตรวจอัลตราซาวด์ จำนวนผู้ที่ทำ CVS และเจาะเลือดสายสะดือในสถาบันต่าง ๆ ที่เข้าร่วมโครงการนี้มีไม่มากพอที่จะนำมาทำการศึกษาจนมีกำลังการทดสอบที่น่าเชื่อถือได้
- โครงการการหาค่าปกติของมาร์คเกอร์ต่าง ๆ ทางอัลตราซาวด์ที่สัมพันธ์กับโลหิตจางของทารกในครรภ์ ถือว่าประสบผลสำเร็จเป็นอย่างมาก ทำให้ได้ค่ามาตรฐานของทารกไทยไว้ประเมินในกรณีทารกมีความเสี่ยง

ประสิทธิภาพของมาร์คเกอร์ต่าง ๆ ทางอัลตราซาวด์ในการแยกทารกโลหิตจางจากโรค Hb Bart's จากทารกปกติ ถือว่าประสบผลสำเร็จเป็นอย่างมากเช่นเดียวกัน ทำให้สามารถทราบและนำมาประยุกต์ใช้ร่วมกันหลาย ๆ พารามิเตอร์ในการช่วยวินิจฉัยทารกโรคฮีโมโกลบินบาร์ท (เช่น ค่าดอพเลอร์เส้นเลือดในสมอง เส้นเลือดม้าม เส้นรอบวงม้าม ความยาวตับเป็นต้น)

Executive Summary

1. **ชื่อโครงการ :** การควบคุมโรคธาลัสซีเมียชนิดรุนแรงด้วยวิธีก่อนคลอด (Prenatal Control of Severe Thalassemia Syndrome) แบ่งเป็นโครงการย่อยดังต่อไปนี้
   - **Project 1:** A comparison of the accuracy of Erythrocyte osmotic fragility test, MCV, and MCH in screening α-thalassemia-1 and β thalassemia carriers *(Supatra Sirichotiyakul, et al)*
   - **Project 2:** Comparison of Accuracy of DCIP, KKU-DCIP and HbE screen Test for Screening of HbE Trait in Pregnant Women *(Chanane Wanapirak, et al)*
   - **Project 3:** Accuracy of α-Thal IC Strip Test in Diagnosis of α-Thalassemia1 Carrier *(Theera Tongsong, et al)*
   - **Project 4 (Main Project): Prenatal Control of Severe Thalassemia *(Theera Tongsong, et al)***
   - **Project 5:** Differentiating affected and unaffected fetuses in pregnancies at risk of Hb Bart's disease in the first half of pregnancies *(Suchaya Luewan, et al)*
   - **Project 6:** Normal Reference Range of Fetal Parameters Used in Evaluation of Fetal Anemia-Associated Hb Bart's Disease *(Fuanglada Tongprasert, et al)*
   - **Project 7: nteractive Multimedia E-learning:** Workshop on Sonographic Diagnosis of Hb Bart's disease and Hydrops Fetalis
   - **Project 8: Textbook:** Prenatal control of severe thalassemia
   - **Project 9:** Pregnancy outcomes among women complicated with thalassemia syndrome *(Suchaya Luewan et al)* (Project นี้เป็น proposal เพิ่มเติมที่นำเสนอเพิ่มในหกเดือนแรกของการเริ่มทำวิจัย ดังที่ได้แจ้งไว้ในรายงานครั้งที่ 1)

2. **ชื่อหัวหน้าโครงการ หน่วยงานที่สังกัด ที่อยู่ หมายเลขโทรศัพท์ โทรสาร และ e-mail**
   - น.พ. ธีระ ทองสง
   - ภาควิชาสูติศาสตร์และนรีเวชวิทยา คณะแพทยศาสตร์ มหาวิทยาลัยเชียงใหม่
   - โทรศัพท์ 053-946429, 053-945552 โทรสาร 053-946112
   - E-mail: ttongson@mail.med.cmu.ac.th
3. **สาขาวิชาที่ทำการวิจัย**
   - เวชศาสตร์มารดาและทารก (Maternal-fetal medicine)
4. **งบประมาณทั้งโครงการ**
   - 7.5 ล้านบาท

5. **ระยะเวลาดำเนินการ**
   - 3 ปี

6. **ภาพรวมของปัญหาที่ทำการวิจัยและโครงการที่ได้ดำเนินการ**

โรคธาลัสซีเมีย (Thalassemia) เป็นกลุ่มโรคผิดปกติทางพันธุกรรมของเม็ดเลือดแดง ก่อให้เกิดพยาธิสภาพแทบทุกอวัยวะของร่างกาย เป็นโรคที่พบได้ชุกชุมมากในประชากรไทย ในเอเชียอาคเนย์ ในประเทศไทยมีผู้ที่มียีนธาลัสซีเมียกว่า 20 ล้านคน สำหรับผู้ที่เป็นโรคธาลัสซีเมียชนิดต่าง ๆ มีสัดส่วนประมาณร้อยละ 1 ของประชากร คือประมาณ 630,000 คน ผู้ที่เป็นโรคมีอาการต่าง ๆ กัน ตั้งแต่โลหิตจางเล็กน้อย ไปจนถึงรุนแรงมาก หรือเสียชีวิตตั้งแต่อยู่ในครรภ์ หรือช่วงสั้น ๆ หลังคลอด สำหรับโรคธาลัสซีเมียที่จัดว่ารุนแรง ซึ่งเป็นปัญหาสำคัญของสาธารณสุขไทย ได้แก่ Hb Bart's disease (ตายคลอดหมด), homozygous β-thalasssemia major (อายุขัยเฉลี่ย 10 ปี) และ β-thalassemia / HbE (อายุขัยเฉลี่ย 30 ปี) โดยประชากรไทยร้อยละ 36.90 เป็นพาหะของโรคธาลัสซีเมียและฮีโมโกลบินผิดปกติที่มีโอกาสมีบุตรเป็นโรคทั้งสามดังกล่าว ส่วนพาหะที่พบมากเป็นอันดับหนึ่งคือ Hb E trait (ร้อยละ 27.9) รองลงมาคือพาหะ α-thalassemia1 และ β-thalassemia ตามลำดับ การตั้งครรภ์ 10,000 รายจะมีโอกาสมีบุตรเป็นโรครุนแรงดังกล่าว 638 ราย ซึ่งนับเป็นจำนวนที่สูงมาก โดยเขตภาคเหนือจะมีความเสี่ยงสูงสุด เนื่องจากเป็นแถบที่มีพาหะของ α-thalassemia1 และ β-thalassemia มากกว่าภาคอื่น ๆ ในแต่ละปีจะมีการคลอดของประชากรไทย 800,000 คน จะมีคู่เสี่ยงต่อการมีบุตรเป็นโรครุนแรงอยู่ 17,012 คู่ และคลอดบุตรเป็นโรครุนแรง 4,253 รายต่อปี ซึ่งค่าดูแลรักษาโดยรวมราว 21,500 ล้านบาท (มีคู่เสี่ยงต่อ β-thalassemia major 826 ราย คลอดบุตรเป็นโรค 207 ราย ค่าใช้จ่ายตลอดอายุขัยมากกว่า 260 ล้านบาท คู่เสี่ยงต่อ β-thalassemia / Hb E 12,853 ราย คลอดบุตรเป็นโรค 3,213 ราย ค่ารักษาตลอดอายุขัยมากกว่า 21,205 ล้านบาท และคู่เสี่ยงต่อ Hb Bart's disease 3,333 ราย คลอดบุตรเป็นโรค 833 ราย ค่ารักษาเกือบ 21 ล้านบาท) นับเป็นภาระกิจใหญ่หลวงของประเทศ จึงได้มีแนวคิดในการป้องกันและควบคุม การควบคุมโดยวิธีคัดกรองและวินิจฉัยก่อนคลอดได้รับการพิสูจน์ว่ามีประสิทธิภาพสูง ซึ่งประกอบด้วยหลักการสำคัญคือ 1) ให้ความรู้ ซึ่งเป็นปัจจัยสำคัญต่อความสำเร็จของโครงการ โดยเน้นความเป็นพาหะ ความเป็นคู่เสี่ยง และความเสี่ยงต่อการมีลูกเป็นโรค 2) การตรวจคัดกรองหาพาหะ โดยเฉพาะอย่างยิ่งในขณะตั้งครรภ์ ซึ่งถ้าสตรีเป็นพาหะ ต้องคัดกรองสามีดูว่าเป็นพาหะด้วยหรือไม่ 3) การให้คำปรึกษาทางพันธุศาสตร์ ให้คู่สมรสเข้าใจถึงความเสี่ยง ธรรมชาติของโรค การหลีกเลี่ยงการมีลูกที่เป็นโรค 4) การวินิจฉัยก่อนคลอด (ในรายที่เป็นคู่เสี่ยง) และ 5) ให้ทางเลือกแก่คู่สมรส ในการตัดสินใจยุติการตั้งครรภ์ หรือเตรียมพร้อมกับการดูแลบุตรที่เป็นโรค เป็นต้น

ในการควบคุมด้วยวิธีก่อนคลอด ยังคงมีความหลากหลาย ในวิธีคัดกรอง วิธีวินิจฉัยพาหะ วิธีวินิจฉัยทารกในครรภ์ บางแนวทางอาศัยการวินิจฉัยทารกในครรภ์ด้วยการวิเคราะห์ดีเอ็นเอ ซึ่งมีความเหมาะสมแตกต่างกันไปตามภูมิภาค ความชุก เศรษฐานะ การวิเคราะห์ดีเอ็นเอทารกมีข้อดีที่วินิจฉัยได้เร็วตั้งแต่อายุครรภ์ 11-14 สัปดาห์ (โดยตรวจจากชิ้นเนื้อรก) หรือ 16-18 สัปดาห์ (ตรวจเซลล์น้ำคร่ำ) แต่มีข้อเสียที่ราคาแพง ต้องการเทคโนโลยีที่ซับซ้อนและไม่สามารถทำได้ในวงกว้างของประเทศ โดยเฉพาะในประเทศกำลังพัฒนา จึงได้มีความ

พยายามค้นหาแนวทางที่ราคาถูก สะดวกกับการประยุกต์ในวงกว้าง ซึ่งประสบการณ์ของโรงพยาบาลมหาราชนครเชียงใหม่ได้รายงานการประยุกต์ในวงกว้างด้วยวิธีราคาถูกซึ่งประสบความสำเร็จอย่างมากโดยไม่ต้องอาศัยเทคนิคการตรวจดีเอ็นเอ แต่ก็ยังคงมีข้อด้อยอยู่บ้างเช่น การวินิจฉัยทารกด้วยการวิเคราะห์เลือดจากสายสะดือถือเป็นการวินิจฉัยค่อนข้างช้า คือ 18-22 สัปดาห์ นอกจากนั้นยังคงมีปัญหาหลายประการที่ต้องการหลักฐานทางวิทยาศาสตร์มาสนับสนุน เช่น แม้ยุทธวิธีดังกล่าวทำให้สามารถวินิจฉัยทารกในครรภ์ได้เป็นจำนวนมาก แต่ก็ไม่ทราบถึงประสิทธิภาพหรือความคุ้มทุนในประยุกต์ใช้ในภูมิภาคอื่น ๆ ที่ความชุกอาจน้อยกว่า เป็นต้น นอกจากนั้น ในประเทศไทยมีแนวทางในการคัดกรองหลากหลาย โดยไม่มีการศึกษาเปรียบเทียบระหว่างวิธีในกลุ่มประชากรเดียวกัน เช่น การคัดกรองหาพาหะ α / β thalassemia มีทั้ง OFT, modified OFT, MCV หรือ MCH เป็นต้น การคัดกรองพาหะ Hb E มีการใช้ DCIP, KKU-DCIP, หรือ CMU-HbE screen test เป็นต้น ถึงแม้ว่าการทดสอบต่าง ๆ ที่ใช้กันต่างเคยมีการศึกษาพิสูจน์แล้วว่ามีประสิทธิภาพ แต่ไม่เคยได้รับการศึกษาเปรียบเทียบกันระหว่างวิธีเหล่านั้นในกลุ่มประชากรเดียวกัน และไม่เคยได้รับการประเมินว่าการทดสอบในวงกว้าง หรือในโลกความเป็นจริงของเวชปฏิบัติที่ไม่ได้กระทำโดยผู้เชี่ยวชาญจะผลดีคงเดิม (reproducible) หรือไม่ กล่าวได้ว่าการทดสอบใดดีที่สุดสำหรับการคัดกรองควรได้รับการประเมินอย่างถี่ถ้วน และการประยุกต์ใช้ทางคลินิกยังต้องมีการปรับเปลี่ยนให้ดีขึ้นไปอีก จนปัจจุบันนี้แม้ว่ามีการพิสูจน์ให้เห็นว่ายุทธวิธีเหล่านี้มีคุณค่าในการค้นหาและป้องกันโรครายใหม่ แต่ประสิทธิภาพที่แท้จริงของยุทธวิธียังไม่ได้รับการพิสูจน์ ทุกรายงานการประยุกต์ใช้ทางคลินิกไม่สามารถบอกได้ว่ามีทารกที่เป็นโรคมากน้อยเพียงใดที่หลุดไปเพราะการตรวจคัดกรองไม่ไวพอ (false negative) เป็นต้น

เพื่อให้ประสบผลสำเร็จในการควบคุมโรคในระดับวงกว้าง จำเป็นต้องศึกษาให้ได้วิธีการทดสอบคัดกรองหรือวินิจฉัยพาหะ ที่เหมาะสมที่สุด ในโครงการนี้จึงต้องการศึกษาเปรียบเทียบการทดสอบคัดกรองที่เป็นที่ยอมรับกันในแง่ความถูกต้อง คือ เปรียบเทียบ OFT กับ MCV และ MCH ด้วยการทดสอบทั้ง 3 ชนิดในผู้ป่วยทุกรายสำหรับการคัดกรองα/β thalassemia (Project 1) และเปรียบเทียบ DCIP, KKU-DCIP และ E-screen test ในการคัดกรองพาหะ Hb E (Project 2)

นอกจากนี้ ในปัจจุบันนี้ยุทธวิธีส่วนมากจะยืนยันการเป็นพาหะของ a-thalassemia1 ด้วยวิธี PCR เป็นหลัก แต่มีข้อเสียตรงที่มีราคาแพง ห้องปฏิบัติการไม่มีความพร้อมสำหรับการประยุกต์ใช้ในวงกว้าง ขณะนี้มีการพัฒนาตรวจพาหะ α-thal1 ด้วยเทคนิค immuno-chromatographic strip ซึ่งราคาถูกกว่ามาก และสะดวกในการใช้กว่าเป็นอย่างมาก เป็นแถบจุ่มสำเร็จรูปที่ไม่ต้องการห้องปฏิบัติการในการตรวจ ในการศึกษานำร่องบ่งชี้ว่าอาจใช้แทนเทคนิค PCR ได้ แต่ยังไม่มีการนำมาประยุกต์ใช้ในทางคลินิก ในโครงการนี้จะทำการศึกษาความถูกต้องของ IC strip ว่ามีความถูกต้องเทียบเท่า PCR หรือไม่ โดยทดสอบจากผู้ป่วยรายเดียวกันควบคู่กันไป (Project 3) ซึ่งถ้ามีความถูกต้องใกล้เคียงกัน จะนำไปประยุกต์เป็นเทคนิคหลักในการยืนยันความเป็นพาหะ α-thal1 ในยุทธวิธีก่อนคลอดต่อไป

ปัญหาอีกประการหนึ่งในเวชปฏิบัติสำหรับครรภ์ที่มีความเสี่ยงต่อการ Hb Bart's disease ซึ่งมีทางเลือกในการวินิจฉัยก่อนคลอดวิธี คือ ใช้เทคนิค invasive (ตรวจชิ้นเนื้อรก เจาะน้ำคร่ำ หรือเจาะสายสะดือทารก) หรือ

ตรวจด้วยอัลตราซาวด์ดูการแสดงออกของภาวะโลหิตจางในทารก (ตั้งแต่ระดับก่อนบวมน้ำ จนถึงระดับบวมน้ำชัดเจน) ซึ่งโดยทั่วไปช่วงกึ่งการตั้งครรภ์มักจะเห็นอาการแสดงที่สะท้อนถึงภาวะโลหิตจาง หากพบจะแนะนำให้ยืนยันด้วยการตรวจวินิจฉัยก่อนคลอดโดยเทคนิค invasive ด้วยวิธีการหลังนี้ทำให้ลดจำนวนการทำเทคนิค invasive ลงไปได้มาก อย่างไรก็ตามการตรวจอัลตราซาวด์จะทำบ่อยเพียงใด และติดตามไปถึงอายุครรภ์เท่าใดจึงจะมั่นใจว่าปกติแน่ ไม่ต้องตรวจซ้ำอีก ในโครงการนี้ (ส่วนหนึ่งของ Project 4) จะทำการตรวจอัลตราซาวด์ติดตามทุกรายที่มีความเสี่ยงนี้และเลือกที่จะไม่ทำเทคนิค invasive ตั้งแต่ต้น เพื่อจะศึกษารูปแบบการแสดงออกทางอัลตราซาวด์ของภาวะโลหิตจางในครรภ์เป็นอย่างไร จะได้มีแนวทางในการหารูปแบบในการตรวจติดตามอัลตราซาวด์อย่างเหมาะสมต่อไป

โครงการนี้มีวัตถุประสงค์ คือค้นหาและทดสอบยุทธวิธี (strategy) **ก่อนคลอดที่ดีที่สุดสำหรับประเทศกำลังพัฒนาในการควบคุมโรคธาลัสซีเมียรุนแรง อย่างมีหลักฐานที่หนักแน่น** (solid evidence) **โดยคำนึงถึงทั้งความถูกต้อง และความคุ้มทุน ขณะนี้ได้จบโครงการนี้ได้ทำให้ทราบถึงข้อดีข้อเด่นของการตรวจคัดกรองชนิดต่าง ๆ และทั้งการวินิจฉัยที่แน่นอนสำหรับทั้งพาหะ และการวินิจฉัยทารก และยังได้ทำการทดสอบประสิทธิภาพของยุทธวิธีดังกล่าว (ดังในผลงานตีพิมพ์ชิ้นที่ 20) ซึ่งได้ทำการทดสอบยุทธิวิธีในการประยุกต์ใช้ทางคลินิกจริง และได้ข้อมูลสำคัญสำหรับการนำไปประยุกต์ใช้กับประชากรไทยต่อไป**

ในโครงการนี้สามารถค้นหาทารกที่มีความเสี่ยงต่อ Hb Bart's disease (คู่เสี่ยงซึ่งจะมีโอกาสทารกเป็นโรคร้อยละ 25) จะมีโครงการย่อย (Project 5) ศึกษามาร์คเกอร์ทางอัลตราซาวด์ของทารกในครรภ์ที่บ่งชี้ภาวะโลหิตจาง หรืออาการแสดงก่อนบวมน้ำ เพื่อจะประเมินมาร์คเกอร์ต่าง ๆ ในแยกทารกชีดหรือไม่ในครรภ์ (ทั้งในรายที่ยุติกการตั้งครรภ์เพราะทราบผลการวินิจฉัยด้วยเทคนิค invasive หรือรายที่เลือกตรวจอัลตราซาวด์เป็นทางหลัก) ซึ่งได้ดำเนินการบรรลุวัตถุประสงค์ด้วยดี ดังที่ได้แสดงผลไว้ในส่วนผลงานการตีพิมพ์

นอกจากนี้ทีมผู้วิจัยจึงจะทำการศึกษาพัฒนามาร์คเกอร์ทางอัลตราซาวด์ที่เป็นปกติ (normative data) ของพารามิเตอร์ต่าง ๆ ในการศึกษาเดียวกันนี้ เพื่อเป็นข้อมูลพื้นฐานสำหรับการประเมินในรายที่มีความเสี่ยงต่อไป เชื่อว่าจะเป็นประโยชน์อย่างยิ่งในการประเมินความถูกต้องของมาร์คเกอร์ทางอัลตราซาวด์ในการตรวจภาวะโลหิตจางในครรภ์ ซึ่งจำเป็นต้องใช้ข้อมูลพื้นฐานของครรภ์ปกติที่ได้มาจากประชากรภูมิภาคเดียวกัน

7. **ผลลัพธ์**: โครงการนี้ได้ดำเนินไปตามที่ได้วางแผนไว้เป็นส่วนใหญ่ แต่ได้มีการดัดแปลงบางโครงการย่อยภายในซึ่งผลลัพธ์ที่สำคัญมีดังนี้

- เปรียบเทียบความถูกต้องในการคัดกรองพาหะ α thalassemia1 / β thalassemia1 ของ OFT, MCV และ MCH พบว่าผลใกล้เคียงกัน แต่ MCV จะให้ผลเหนือกว่าเล็กน้อย และมีความสะดวกจึงได้เลือกให้เป็นเทคนิคลำดับแรกของการคัดกรอง alpha-/beta thalassemia
- เปรียบเทียบความถูกต้องในการคัดกรองพาหะ Hb E พบว่า E-Screen (CMU-E screen) Test ดีที่สุด มีความไวถึงร้อยละ 100 และผลบวกลวงต่ำมาก จึงแนะนำให้เป็นเทคนิคลำดับแรกของการคัดกรอง HbE
- ความถูกต้องในการวินิจฉัยพาหะ α thalassemia1 ของ alpha-thal-IC strip มีความไวสูงมาก แต่มีผลบวกลวง อยู่มากกว่าร้อยละ 10 แม้จะเป็นการทดสอบที่สะดวก ใช้ได้แพร่หลาย ไม่จำเป็นต้องมี

เครื่องมือการตรวจราคาแพงแบบ PCR แต่ก็ยังไม่สามารถนำมาใช้ทดแทน PCR ได้ แต่อาจใช้ IC-strip เป็น option หรือ secondary screening เพื่อคัดเลือกบางรายไปตรวจ PCR หรือในรายที่ให้ผลบวกให้ตรวจอัลตราซาวด์แทนการตรวจ PCR (ไม่ต้องเจาะเลือดสายสะดือ)

- การศึกษาประสิทธิภาพของยุทธวิธีก่อนคลอดในการวินิจฉัยธาลัสซีเมียก่อนคลอด ซึ่งเป็นการศึกษาหลายสถาบัน (multicenter) หลังจากที่ได้พยายามศึกษาวิธีการคัดกรองใน project 1-3 แล้ว ได้นำวิธีการที่คัดกรองและวินิจฉัยก่อนคลอดมาประยุกต์และประเมินผล ซึ่งนับว่าได้ผลดี ดังที่ได้แสดงใน project 4 (ผลงานตีพิมพ์ชิ้นที่ 20)
- การศึกษาถึงภาวะแทรกซ้อนต่าง ๆ ของหัตถการเพื่อการวินิจฉัยก่อนคลอด ไม่สามารถทำได้ตามที่ตั้งใจไว้ตั้งแต่ต้น เนื่องจากการศึกษาในหลายสถาบัน หลีกเลี่ยงการเจาะเลือดสายสะดือ หรือ CVS ไปเป็นการเจาะน้ำคร่ำ หรือตรวจอัลตราซาวด์ จำนวนผู้ที่ทำ CVS และเจาะเลือดสายสะดือในสถาบันต่าง ๆ ที่เข้าร่วมโครงการนี้มีไม่มากพอที่จะนำมาทำการศึกษาจนมีกำลังการทดสอบที่น่าเชื่อถือได้
- โครงการการหาค่าปกติของมาร์คเกอร์ต่าง ๆ ทางอัลตราซาวด์ที่สัมพันธ์กับโลหิตจางของทารกในครรภ์ ถือว่าประสบผลสำเร็จเป็นอย่างมาก ทำให้ได้ค่ามาตรฐานของทารกไทยไว้ประเมินในกรณีทารกมีความเสี่ยง
- ประสิทธิภาพของมาร์คเกอร์ต่าง ๆ ทางอัลตราซาวด์ในการแยกทารกโลหิตจางจากโรค Hb Bart's จากทารกปกติ ถือว่าประสบผลสำเร็จเป็นอย่างมากเช่นเดียวกัน ทำให้สามารถทราบและนำมาประยุกต์ใช้ร่วมกันหลาย ๆ พารามิเตอร์ ในการช่วยวินิจฉัยทารกโรคฮีโมโกลบินบาร์ท (เช่น ค่าดอพเพลอร์เส้นเลือดในสมอง เส้นเลือดม้าม เส้นรอบวงม้าม ความยาวตับเป็นต้น)

Output ที่ได้จากโครงการ

1 ผลงานตีพิมพ์ในวารสารวิชาการนานาชาติ

โครงการนี้ได้สร้างงานวิจัยที่ตีพิมพ์ในวารสารนานาชาติ (ที่อยู่ในฐานข้อมูล Scopus) จำนวน 17 เรื่อง และอยู่ระหว่างรอตีพิมพ์ในวารสารนานาชาติ (ที่อยู่ในฐานข้อมูล Scopus) อีก 3 เรื่อง จึงคาดว่าจะมีผลงานตีพิมพ์ดังกล่าว 20 เรื่อง มี impact factor รวมประมาณ 32 ผลงานวิจัยที่ตีพิมพ์มีดังนี้

1 Traisrisilp K, Luewan S, Tongsong T. Pregnancy outcomes in women complicated by thalassemia syndrome at Maharaj Nakorn Chiang Mai Hospital. Arch Gynecol Obstet 2009; 279(5):685-689. *Impact factor: 0.912*

2 Tongsong T, Srisupundit K, Luewan S. Outcomes of pregnancies affected by hemoglobin H disease. Int J Gynaecol Obstet 2009; 104(3):206-208. *Impact factor: 1.408*

3 Luewan S, Srisupundit K, Tongsong T. Outcomes of pregnancies complicated by beta-thalassemia/hemoglobin E disease. Int J Gynaecol Obstet 2009; 104(3):203-205. *Impact factor: 1.408*

4 Tongsong T, Tongprasert F, Srisupundit K, Luewan S. High fetal splenic artery peak velocity in fetuses with hemoglobin Bart disease: a preliminary study. J Ultrasound Med 2009; 28(1):13-18. *Impact factor: 1.181*

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6 Wanapirak C, Sirichotiyakul S, Luewan S, Srisupundit K, Tongsong T. Comparison of the accuracy of dichlorophenolindophenol (DCIP), modified DCIP, and hemoglobin E tests to screen for the HbE trait in pregnant women. Int J Gynaecol Obstet 2009; 107(1):59-60. *Impact factor: 1.408*

7 Pranpanus S, Sirichotiyakul S, Srisupundit K, Tongsong T. Sensitivity and specificity of mean corpuscular hemoglobin (MCH): for screening alpha-thalassemia-1 trait and beta-thalassemia trait. J Med Assoc Thai 2009; 92(6):739-743. *Impact factor: 0.4*

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10 Udomwan P, Luewan S, Tongsong T. Fetal aortic arch measurements at 14 to 40 weeks' gestation derived by spatiotemporal image correlation volume data sets. J Ultrasound Med 2009; 28(12):1651-1656. *Impact factor: 1.181*

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12 Srisupundit K, Piyamongkol W, Tongprasert F, Luewan S, Tongsong T. Reference range of fetal splenic circumference from 14 to 40 weeks of gestation. Arch Gynecol Obstet 2010 Feb 5. *Impact factor: 0.912*

13 Tongsong T, Tongprasert F, Srisupundit K, Luewan S. Venous Doppler Studies in Low Output and High Output Hydrops Fetalis. American Journal of Obstetrics and Gynecology Am J Obstet Gynecol. 2010 Nov;203(5):488. *Impact factor: 3.278*

14 Tongprasert F, Srisupundit K, Luewan S, Tongsong T. Reference Range of Fetal Liver Length from 14-40 Weeks of Gestation. J Clin Ultrasound 2010 Nov 12 *Impact factor: 0.91*

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สรุปโครงการนี้ได้รับการตีพิมพ์ (เฉพาะที่อยู่ในฐานข้อมูล scopus) **ทั้งหมด** 20 **เรื่อง (โดยมี น.พ. ธีระ ทองสง เป็น** corresponding author **ทั้ง** 19 **เรื่อง, เรื่องที่** 19 **น.พ.ชเนนทร์ วนาภิรักษ์ เป็น** corresponding author)

2 หนังสือ Textbook: Prenatal control of severe thalassemia

ในโครงการนี้ ทีมกลุ่มวิจัยได้ผลิตหนังสือการควบคุมโรคธาลัสซีเมียด้วยยุทธวิธีก่อนคลอด ซึ่งได้เขียนจากการทบทวนวารสารทางการแพทย์ และหลักฐานเชิงประจักษ์จากงานวิจัยต่าง ๆ ที่กลุ่มวิจัยได้ศึกษาค้นคว้ามา เชื่อว่าหนังสือเล่มนี้ได้เป็นแนวทางปฏิบัติทางคลินิกเกี่ยวกับการควบคุมโรคธาลัสซีเมียก่อนคลอดในประชากรไทย บนพื้นฐานความรู้ที่มีหลักฐาน (evidenced-based) ทันสมัย สอดคล้องกับเวชปฏิบัติจริง รูปแบบตำราตามมาตรฐานสากล และมีการแพร่หลายไปตามโรงพยาบาลต่าง ๆ ที่มีการตรวจคัดกรองและควบคุมโรคธาลัสซีเมีย โดยแพร่กระจายผ่านผู้มาร่วมประชุมวิชาการในโครงการ และโรงพยาบาลต่าง ๆ ที่อยู่ในโครงการวิจัย มีความคาดหวังว่าหนังสือนี้จะมีประโยชน์แก่แพทย์และเจ้าหน้าที่ที่ต้องให้บริการงานดังกล่าวในระดับปฏิบัติงาน และเป็นหลักฐานอ้างอิงสำหรับการวางนโยบายในระดับต่าง ๆ มีผู้นิพนธ์ร่วมกัน 9 ท่าน มีความหนา 230 หน้า A4 มีเนื้อหาประกอบด้วย

บทที่ 1 โรคธาลัสซีเมีย *โดยนักวิจัยในโครงการ: สุพัตรา ศิริโชติยะกุล*

บทที่ 2 โรคธาลัสซีเมียในเวชปฏิบัติ *โดยนักวิจัยในโครงการ*: พิมพ์ลักษณ์ เจริญขวัญ

บทที่ 3 การควบคุมธาลัสซีเมียจากงานวิจัยสู่การประยุกต์ทางคลินิกอย่างคุ้มค่า *โดยนักวิจัยในโครงการ: ธีระ ทองสง, ชเนนทร์ วนาภิรักษ์, สุพัตรา ศิริโชติยะกุล*

บทที่ 4 การตรวจคัดกรองและตรวจยืนยันพาหะธาลัสซีเมีย *โดยนักวิจัยในโครงการ: สุพัตรา ศิริโชติยะกุล, สุชยา ลือวรรณ*

บทที่ 5 การวินิจฉัยก่อนคลอดของโรคธาลัสซีเมียชนิดรุนแรง: ภาพรวม *โดยนักวิจัยในโครงการ: สุพัตรา ศิริโชติยะกุล*

บทที่ 6 การวินิจฉัยก่อนคลอดของโรคธาลัสซีเมียชนิดรุนแรง: การตัดชิ้นเนื้อรก *โดยนักวิจัยในโครงการ: สุพัตรา ศิริโชติยะกุล*

บทที่ 7 การวินิจฉัยก่อนคลอดของโรคธาลัสซีเมียชนิดรุนแรง: การเจาะดูดน้ำคร่ำ *โดยนักวิจัยในโครงการ: ชเนนทร์ วนาภิรักษ์*

บทที่ 8 การวินิจฉัยก่อนคลอดของโรคธาลัสซีเมียชนิดรุนแรง: การเจาะเลือดสายสะดือ *โดยนักวิจัยในโครงการ: ชเนนทร์ วนาภิรักษ์, สุพัตรา ศิริโชติยะกุล*

บทที่ 9 การวินิจฉัยทารกในครรภ์: การวินิจฉัยโรคฮีโมโกลบินบาร์ทด้วยอัลตราซาวด์ *โดยนักวิจัยในโครงการ: ชเนนทร์ วนาภิรักษ์, สุพัตรา ศิริโชติยะกุล, ธีระ ทองสง*

บทที่ 10 การวินิจฉัยทารกในครรภ์: การตรวจทางห้องปฏิบัติการ *โดยนักวิจัยในโครงการ: พิมพ์ลักษณ์ เจริญขวัญ*

บทที่ 11 การวินิจฉัยโรคทางพันธุกรรมระยะก่อนการฝังตัว *โดยนักวิจัยในโครงการ: วีรวิทย์ ปิยะมงคล*

บทที่ 12 ความสำคัญของโรคธาลัสซีเมียต่อการสาธารณสุขของประเทศไทย *โดยนักวิจัยในโครงการ: สุพัตรา ศิริโชติยะกุล, พรรณี ศิริวรรธนาภา*

บทที่ 13 การควบคุมและป้องกันโรคธาลัสซีเมียจากเริ่มต้นจนปัจจุบันและประเด็นในอนาคต *โดยนักวิจัยในโครงการ: ชเนนทร์ วนาภิรักษ์*

บทที่ 14 เซลล์ต้นกำเนิดการรักษาที่ท้าทาย *โดยนักวิจัยในโครงการ: สุพัตรา ศิริโชติยะกุล, เฟื่องลดา ทองประเสริฐ*

บทที่ 15 การให้คำปรึกษาทางพันธุศาสตร์เกี่ยวกับโรคโลหิตจางธาลัสซีเมีย *โดยนักวิจัยในโครงการ:* ชเนนทร์ วนาภิรักษ์, เกษมศรี ศรีสุพรรณดิฐ

3 Interactive Multimedia E-learning (E-Book): Workshop on Sonographic Diagnosis of Hb Bart's disease and Hydrops Fetalis

ทีมวิจัยของกลุ่มได้ร่วมมือกันพัฒนาสื่อการเรียนรู้แบบมัลติมีเดียตามแผนการที่วางไว้ ซึ่งมีนักพัฒนาได้แก่ ร.ศ. นพ. ชเนนทร์ วนาภิรักษ์, ร.ศ. พญ. สุพัตรา ศิริโชติยะกุล,อ.พญ. เกษมศรี ศรีสุพรรณดิฐ, อ.พญ. สุชยา ลือวรรณ, ศ.นพ. ธีระ ทองสง

โดยสื่อการเรียนรู้นี้เน้นพัฒนาทักษะในการวินิจฉัยโรคฮีโมโกลบินบาร์ท (ซึ่งเป็นธาลัสซีเมียชนิดรุนแรงที่เป็นปัญหากับสตรีตั้งครรภ์ของไทย โดยเฉพาะภาคเหนือตอนบน ปัญหาสำคัญที่ผ่านมาคือ ผู้ป่วยที่ตั้งครรภ์ทารกบวมน้ำจากฮีโมโกลบินบาร์ทไม่ได้รับการวินิจฉัยก่อนคลอดด้วยวิธีการตรวจเลือดทารก หรือวิธีการอื่น ๆ รวมทั้งไม่ได้รับการวินิจฉัยแม้แต่ด้วยคลื่นเสียงความถี่สูงในระยะแรก ๆ ทั้งที่ปัจจุบันนี้เครื่องตรวจคลื่นเสียงความถี่สูงมีใช้แพร่หลายในประเทศไทย แต่ประสบการณ์ในการวินิจฉัยภาวะทารกบวมน้ำด้วยคลื่นเสียงความถี่สูงนับว่ายังมีขีดจำกัดมาก จากเหตุผลสำคัญคือคลื่นเสียงความถี่สูงสามารถวินิจฉัยภาวะทารกบวมน้ำได้เร็วกว่าที่เคยเข้าใจกันมาในอดีต เป็นเทคนิคการวินิจฉัยที่ไม่เจ็บตัว (non-invasive) ไม่เพิ่มความเสี่ยงใด ๆ แก่ทารกและมารดา และเป็นเทคนิคราคาถูกที่มีใช้ทั่วไป

วัตถุประสงค์ของสื่อนี้เพื่อให้แพทย์ได้เพิ่มทักษะในการวินิจฉัยให้เร็วขึ้น โดยคาดหวังว่าสูติแพทย์และรังสีแพทย์ทั่วไปที่ตรวจอัลตราซาวด์ทางสูติกรรมจะสามารถวินิจฉัยทารกบวมน้ำจากโรคฮีโมโกลบินบาร์ทตั้งแต่ระยะเริ่มแรกได้ด้วยความมั่นใจ ลดความจำเป็นในการตรวจหาพาหะ alpha-thalassemia-1 ด้วยเทคนิคราคาแพง (PCR) และลดความจำเป็นในการเจาะเลือดสายสะดือทารก และรวมถึงลดค่าใช้จ่ายในโครงการควบคุมและป้องกันธาลัสซีเมียชนิดรุนแรงด้วยยุทธวิธีก่อนคลอดลงได้ในปริมาณมาก

เมื่อชุดอินเตอร์แอคทีฟมัลติมีเดีย ทางทีมงานได้แพร่กระจายผ่านผู้มาร่วมประชุมวิชาการในโครงการ และโรงพยาบาลต่าง ๆ ที่อยู่ในโครงการวิจัย มีความคาดหวังว่าหนังสือนี้จะมีประโยชน์แก่แพทย์และเจ้าหน้าที่ที่ต้องให้บริการงานดังกล่าวในระดับปฏิบัติงาน

เนื้อหาหลักเน้นการตรวจอัลตราซาวด์มาร์กเกอร์ต่าง ๆ ในการวินิจฉัยทารกที่เป็นโรคฮีโมโกลบินบาร์ททั้งที่บวมน้ำ และยังไม่บวมน้ำ โดยผ่านทางวิโดโอคลิปอัลตราซาวด์คุณภาพสูง 150 คลิป (ภาพอัลตราซาวด์อีก 400 ภาพ) มีคำบรรยายประกอบ วิดีโอสามารถควบคุมการเล่น หรือปรับทีละเฟรมเพื่อให้ได้วัดสัดส่วนที่ถูกต้องจากเฟรมที่ดีที่สุด เป็นต้น แต่ละคลิปสามารถหยุดคลิปดู label และ overlay ได้ อีกทั้งมี workshops ให้ฝึกปฏิบัติเอง และมีภาพการวัดที่ถูกต้องมาเฉลยด้วย

4. การนำผลงานไปใช้ประโยชน์ในเชิงสาธารณะ

- ประโยชน์ที่เกิดขึ้นแก่วงการวิจัย:
    - มีผลเพิ่มจำนวนนักวิจัยรุ่นใหม่ที่มีผลงานระดับนานาชาติมากขึ้นเป็นจำนวนมาก ดังตัวอย่างที่เห็นได้ว่านักวิจัยในกลุ่มมีผลงานวิจัยเพิ่มขึ้นอย่างมาก
- ผลประโยชน์ในเชิงสาธารณะ
    - งานวิจัยด้านการวินิจฉัยก่อนคลอดเกี่ยวกับโรคธาลัสซีเมียชนิดรุนแรง (ด้วยความร่วมมือของทีมงานเวชศาสตร์มารดาและทารก และห้องปฏิบัติการกุมารเวชศาสตร์) ศึกษาค้นคว้ายุทธวิธีในการคัดกรอง และวินิจฉัยก่อนคลอด ซึ่งมีผลทำให้อุบัติการณ์ของโรคธาลัสซีเมียรายใหม่ในภูมิภาคแถบนี้ลดลงอย่างมากในปัจจุบัน จากการศึกษาเทคนิคในการคัดกรอง การทำการวินิจฉัยก่อนคลอด (โดยเฉพาะวิธีการเจาะเลือดสายสะดือทารก) โดยเฉพาะอย่างยิ่งปัญหาโรคธาลัสซีเมียชนิดรุนแรง ซึ่งมีอุบัติการณ์ที่ลดลงอย่างเป็นรูปธรรม
    - งานวิจัยด้านคลื่นเสียงความถี่สูงของทารกในครรภ์ ยังผลให้ความพิการโดยกำเนิดของทารกในครรภ์ โดยเฉพาะด้านหัวใจทารก และโรคฮีโมโกลบินบาร์ท ส่วนใหญ่ได้รับการวินิจฉัยและได้รับการดูแลเหมาะสมตั้งแต่อยู่ในครรภ์ และงานวิจัยจำนวนมากได้กลายเป็นพื้นฐานสำคัญของการพัฒนาสาขาวิชานี้ในประเทศไทย โดยเฉพาะอย่างยิ่งการศึกษาค้นคว้าเกี่ยวกับทารกบวมน้ำจากโรคฮีโมโกลบินบาร์ท (ซึ่งเป็นความผิดปกติของทารกในครรภ์ที่พบบ่อยมากที่สุด โดยเฉพาะแถบภาคเหนือของประเทศไทย และเป็นอันตรายแก่มารดาได้มาก เช่น ความดันโลหิตสูงขณะตั้งครรภ์ คลอดยาก ตกเลือดหลังคลอด เป็นต้น) งานการศึกษาวิจัยนี้ทำให้วินิจฉัยโรคดังกล่าวได้ตั้งแต่ระยะแรกของการตั้งครรภ์ได้มาก ทำให้ทารกภาวะบวมน้ำจากสาเหตุดังกล่าวลดลงได้อย่างมาก
- ผลประโยชน์ในเชิงนโยบาย
    - จากผลงานวิจัยยุทธวิธีในการควบคุมโรคธาลัสซีเมีย (Prenatal control of severe thalassemia: prenatal control) และวิจัยอื่น ๆ ที่เกี่ยวข้อง ได้กลายเป็นต้นแบบ หรือแหล่งอ้างอิงอย่างมีหลักฐานทางวิทยาศาสตร์ สำหรับกระทรวงสาธารณสุขในการนำไปประยุกต์ใช้ระดับประเทศ และเป็นที่อ้างอิงในการพัฒนานโยบายในการควรคุมโรคนี้ในทุกระดับ คาดว่าผลงานวิจัยเชิงคลินิกของทีมงานได้มีผลต่อระดับชาติในเชิงนโยบาย

5. รางวัลที่ได้รับระหว่างรับทุนส่งเสริมกลุ่มวิจัย

- รางวัลโครงการวิจัยเด่น สกว. ประจำปี 2552
- รางวัลระดับนานาชาติ: รางวัลวิจัยทางคลินิกดีเด่นจากประเทศกำลังพัฒนา ของสมาพันธ์นานาชาติทางสูติศาสตร์และนรีเวชวิทยา (FIGO: International Federation of Gynecology & Obstetrics) (JJ Scirra IJGO Prize Award Honorable Mention 2009) จากผลงานวิจัย Luewan S, Srisupundit K, Tongsong T. Outcomes of pregnancies complicated by beta-thalassemia/ hemoglobin E disease. Int J Gynecol Obstet 2009;104:203-5.

6. นักวิจัยในโครงการได้รับรางวัลหรือทุนวิจัยอื่นในระหว่างรับทุนส่งเสริมกลุ่มวิจัย

- รางวัลโครงการวิจัยเด่น สกว. ประจำปี 2552
- ชื่อนักวิจัย: ศ.นพ. ธีระ ทองสง
  - ได้รับรางวัล นักวิจัยดีเด่นประจำปี 2551 มหาวิทยาลัยเชียงใหม่ (รางวัลมหาวิทยาลัยเชียงใหม่ รางวัลช้างทองคำ: เป็นรางวัลใหญ่ที่สุดของมหาวิทยาลัยเชียงใหม่สำหรับนักวิจัย ซึ่งมีปีละ 1 รางวัล)
- ชื่อนักวิจัย: รศ.นพ. ชเนนทร์ วนาภิรักษ์
  - ได้รับรางวัล นักวิจัยดีเด่นประจำปี 2552 มหาวิทยาลัยเชียงใหม่ (รางวัลมหาวิทยาลัยเชียงใหม่ รางวัลช้างทองคำ: เป็นรางวัลใหญ่ที่สุดของมหาวิทยาลัยเชียงใหม่สำหรับนักวิจัย ซึ่งมีปีละ 1 รางวัล)
- ชื่อนักวิจัย: รศ.นพ. วีรวิทย์ ปิยะมงคล
  - ได้รับทุนวิจัยจากสำนักงานคณะกรรมการวิจัยแห่งชาติ (วช): เรื่องการตรวจวินิจฉัยก่อนการฝังตัวสำหรับโรคเบต้าธาลัสซีเมียและกลุ่มเลือด HLA ปี 2551-2553
- ชื่อนักวิจัย: รศ.พญ. สุพัตรา ศิริโชติยะกุล
  - ทุนวิจัยองค์ความรู้ใหม่ที่เป็นพื้นฐานต่อการพัฒนา (วุฒิเมธีวิจัย สกว.) เมื่อ 1 พฤษภาคม พ.ศ. 2551 – 30 เมษายน พ.ศ. 2554 สัญญาเลขที่ RSA5180013
  - รางวัลผลงานวิชาการดีเด่นประเภทการนำเสนอแบบโปสเตอร์ เนื่องในการประชุมสัมมนาวิชาการธาลัสซีเมียแห่งชาติ ครั้งที่ 15 วันที่ 22-24 เมษายน พ.ศ. 2552 ณ โรงแรมเจริญศรีแกรนด์รอยัล จ. อุดรธานี
- ชื่อนักวิจัย: ผศ.พญ. เฟื่องลดา ทองประเสริฐ
  - ได้รับทุนวิจัยจากสกว.: ทุนพัฒนาศักยภาพในการทำงานวิจัยของอาจารย์รุ่นใหม่ ปี 2552
- ชื่อนักวิจัย: อาจารย์ พญ. เกษมศรี ศรีสุพรรณดิฐ
  - ได้รับทุนวิจัยจากสกว.: ทุนพัฒนาศักยภาพในการทำงานวิจัยของอาจารย์รุ่นใหม่ปี 2551
- ชื่อนักวิจัย: อาจารย์ พญ. สุชยา ลือวรรณ
  - ได้รับทุนวิจัยจากสกว.: ทุนพัฒนาศักยภาพในการทำงานวิจัยของอาจารย์รุ่นใหม่ปี 2553

- ชื่อนักวิจัย: อาจารย์ พญ. สุชยา ลือวรรณ, อาจารย์ พญ. เกษมศรี ศรีสุพรรณดิฐ, ศ.นพ. ธีระ ทองสง ได้รับรางวัลระดับนานาชาติ: รางวัลวิจัยทางคลินิกดีเด่นจากประเทศกำลังพัฒนา ของสมาพันธ์นานาชาติทางสูติศาสตร์และนรีเวชวิทยา (FIGO: International Federation of Gynecology & Obstetrics) (JJ Scirra IJGO Prize Award Honorable Mention 2009) จากผลงานวิจัย Luewan S, Srisupundit K, Tongsong T. Outcomes of pregnancies complicated by beta-thalassemia/ hemoglobin E disease. Int J Gynecol Obstet 2009;104:203-5.

จึงเรียนมาเพื่อโปรดพิจารณา
ขอแสดงความนับถือ

ศาสตราจารย์นายแพทย์ธีระ ทองสง
ภาควิชาสูติศาสตร์และนรีเวชวิทยา
คณะแพทยศาสตร์ มหาวิทยาลัยเชียงใหม่

ภาคผนวก

ผลงานตีพิมพ์จากโครงการ Prenatal Control of Severe Thalassemia

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ORIGINAL ARTICLE

Pregnancy outcomes in women complicated by thalassemia syndrome at Maharaj Nakorn Chiang Mai Hospital

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Abstract
Objective To determine the maternal and fetal outcomes of women complicated with thalassemia syndrome.
Study design Retrospective descriptive study.
Materials and methods The database of Maternal-Fetal Medicine unit and medical records, between January 2001 and April 2008, were reviewed to search for pregnant women complicated with thalassemia syndrome and medical records were reviewed for patient's baseline characteristics and pregnancy outcomes. The inclusion criteria consisted of (1) pregnant women diagnosed for thalassemia syndrome by hematologist either during or before pregnancy based on hemoglobin typing, (2) attending the antenatal care clinic and delivery at Maharaj Nakorn Chiang Mai hospital, and (3) available data of pregnancy outcomes.
Results During the study period, 80 pregnant women with thalassemia syndrome were recruited, including 52 (65%) cases of HbH disease, 23 (28.8%) cases of beta-thalassemia/HbE disease, 2 cases of AE Bart's disease, 2 cases of EF Bart's disease and 1 case of beta-thalassemia major. Excluding 2 twin pregnancies and 1 case with beta-thalassemia major, 77 were available for analysis of the outcomes. The mean gestational age (±SD) at delivery was 37.40 (±2.6) weeks, range 27–42 weeks. Twenty-five (32.5%) had delivery by cesarean section and the remainder had successful vaginal delivery. Fetal growth restriction was found in 21 cases (27.3%), 16 (20.8%) had preterm births and the rate of low birth weight (<2,500 g) was 44.1%. Regard to the type of thalassemia, baseline hemoglobin levels and mean birth weight of women with beta-thal/Hb E was significantly lower than the levels of those with Hb H disease.
Conclusion This series indicates that, in spite of an attempt to keep hemoglobin levels above 7.0 g/dl, pregnancy with thalassemia is likely to be associated with an increased rate of fetal growth restriction, preterm birth and low birth weight.

Keywords Anemia · Pregnancy outcomes · Thalassemia

Introduction

Thalassemia is one of the most common genetic causes of anemia, especially in South East Asia. In our pregnant population, northern part of Thailand, overall prevalence of thalassemia trait may be as high as 25.4% which were classified as follows: alpha-thalassemia-1 (SEA type) trait 6.6%, beta-thalassemia trait 3.7%, hemoglobin (Hb) E trait 11.6%, homozygous HbE 0.8% [1]. However, fertility is usually compromised in patients with thalassemia syndrome; therefore, pregnancy with this disorder is rare. Nevertheless, with medical advances in taking care of these patients, such pregnancies have been encountered more often lately.

Pregnant women with thalassemia syndrome usually suffer from anemia with Hb levels of 7–10 g/dl and variable degrees of splenomegaly [2, 3]. Consequent hypoxia and massive tissue iron deposition may lead to concomitant cardiac, hepatic, and endocrine system failure [4]. The physiologic changes during pregnancy will further increase the severity of anemia in the mother, which could affect pregnancy outcome as well as clinical symptoms [5]. These pregnancies may be associated with a high rate of obstetric complications, especially intrauterine fetal growth restriction

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and preterm labor, attributed to low hemoglobin levels during gestation leading to fetal hypoxia [6, 7].

In Thailand, beta-thalassemia major is unlikely to complicate pregnancy because of its severity and fertility impairment. The main problems encountered during pregnancy are confined to beta-thalassemia/HbE and HbH disease in most cases. HbE (β-26 glutamine → lysine) is the most common hemoglobin variant in Thailand. HbE trait may not be of clinical significance, but interaction of HbE and thalassemia (beta-thalassemia/HbE disease) produces variable phenotype [8] and this is common hemolytic anemia in Southeast Asia. Manifestations of this disorder include refractory anemia, splenomegaly and sometimes, unexplained jaundice.

Hemoglobin H (Hb H) disease is one of the major thalassemic diseases, and it predominantly affects Southeast Asia, the Middle East, Greece, and Cyprus [9]. The loss of three α-genes results in a severe imbalance in the production of globin chains, and the beta-globins produced in excess form Hb H. The clinical manifestations of Hb H disease vary from minimal symptoms to severe chronic hemolytic anemia.

To date, only few small series focusing on pregnancy outcomes among the women complicated with thalassemia syndrome have been reported. Moreover, based on such series, the effect of the disease on pregnancy outcomes has been controversial. Some studies demonstrated favorable outcome with intensive treatment [4, 10, 11], whereas the others showed marked impact on adverse outcomes [12, 13]. Therefore, a large study on this issue is still required. The objective of this study was to determine the maternal and fetal outcomes of women complicated with thalassemia syndrome.

Materials and methods

This is a retrospective descriptive study based on the database of Maternal-Fetal Medicine unit, Department of Obstetrics and Gynecology, Chiang Mai University, and reviewing the medical records of the patients from January 2001 to April 2008. The inclusion criteria consisted of (1) pregnant women diagnosed for thalassemia syndrome by hematologist either during or before pregnancy based on hemoglobin typing, (2) attending the antenatal care clinic and delivery at Maharaj Nakorn Chiang Mai hospital, and (3) available data of pregnancy outcomes.

Patients with thalassemia were usually followed up regularly and were screened for hemoglobin/hematocrit, hepatitis B and human immunodeficiency virus, liver function evaluation, and echocardiography. They were taken care at high risk clinic by both obstetricians and hematologist. Generally, these patients were managed to maintain their Hb levels exceeding 7.0 g/dl. Gestational age was established at first visit, either by clinical estimation or ultrasound dating. The patients were followed up for fetal growth, maternal weight gain, blood pressure measurement, and fetal heart rate documentation. Hb levels are assessed routinely every 2–3 weeks, and baseline ultrasonographic evaluation of fetal growth at mid-pregnancy and serial examinations in case of clinical suspicion for fetal growth restriction. Definitions of pregnancy outcomes were as follows: stillbirth, death of fetus in utero after 20 weeks of gestation, premature birth (preterm), live birth before 37 weeks of gestation, low birth weight, birth weight of less than 2,500 g, and fetal growth restriction (FGR), birth weight below the tenth percentile of the normal growth curve. Fetal surveillance at viable stage was performed as high risk pregnancies. The maternal records were reviewed for maternal age, parity, baseline Hb at first visit and at delivery, number of blood transfusions, Hb electrophoresis results, history of splenomegaly. In addition, obstetric complications like placenta previa, abruptio placentae, preterm labor, previous cesarean, and hypertensive disorders of pregnancy, the mode of delivery, and postpartum complications such metritis and postpartum hemorrhage were also reviewed and recorded. The neonatal records were reviewed for gestational age, birth weight, Apgar scores at 1 and 5 min, fetal growth restriction, congenital anomalies. The main outcomes of measure included, rate of fetuses with growth restriction, preterm birth, low birth weight, Apgar scores and maternal complications such as pre-eclampsia, postpartum hemorrhage, etc. This study was conducted with approval of the Research Ethics Committee 3, Faculty of Medicine Chiang Mai University.

Statistical analysis

The data were analyzed using the statistical package for the social sciences (SPSS). Descriptive data were presented as percentage, means and standard deviation (SD).

Results

During the study period, 82 pregnant women with thalassemia syndrome were diagnosed; however, 2 cases were excluded because of no available final outcomes. The remaining 80 cases included 1 case of thalassemia major (beta-thalassemia major) and 79 cases of thalassemia intermedia including 52 cases (65%) of HbH disease, 23 cases (28.8%) of beta-thalassemia/HbE disease, 2 cases of AE Bart's disease and 2 cases of EF Bart's disease. Seventy-eight (97.5%) pregnancies were singleton and two were twin pregnancies. Nine cases had history of splenectomy before pregnancy. The mean (±SD) maternal age was

28.23 (±5.9) years, range 18–42 years. Most of them (63.8%) were nulliparous. Most of them (76.3%) were living in Chiang Mai province and the remainder lived in the surrounding areas, northern part of Thailand. More than half (56.8%) of the patients had occupation of agriculture or employee. Nearly 90% attended antenatal care clinic at least four times.

To assess pregnancy outcomes, three were excluded due to twin pregnancies and one was beta-thalassemia major. The remaining 77 of thalassemia intermedia were available for analysis of the outcomes.

The mean gestational age (±SD) at delivery was 37.40 (±2.6) weeks, range 27–42 weeks. Mean (±SD) hemoglobin levels at first visit was 8.2 (±2.1) g/dl, range 3.8–12.2 g/dl and mean (±SD) hemoglobin levels at the time of delivery was 7.2 (±2.9) g/dl, range 3.2–11.2 g/dl. The mean birth weight (±SD) was 2,527.14 ± 591 g, range 490–4,050 g.

Twenty-five (32.5%) had delivery by cesarean section and the remainder had successful vaginal delivery. Maternal complications during pregnancy are summarized in the Table 1 and fetal outcomes are presented in Table 2. Notably, fetal growth restriction was found in 21 cases (27.3%), 16 (20.8%) had preterm births and the rate of low birth weight (<2,500 g) was 44.1%.

When compared to Hb H disease, women with beta-thal/Hb E disease had significantly lower baseline hemoglobin levels (6.99 vs. 8.3 gm/dl, $P < 0.05$) and needed more number of blood transfusions (1.69 vs. 0.69 times, $P < 0.05$). However, the hemoglobin levels at the time of delivery were not significantly different. Birth weight of the newborn of mother with beta-thal/Hb E had a tendency to be less than that in those with Hb H disease, but not statistically significant. (Table 3) Likewise, the prevalence of fetal growth restriction and preterm birth seemed to be higher in group of beta-thal/Hb E disease, but not reached statistical significance.

Table 1 Maternal Complications

Complications	Number	Percent
Medical complications		
Diabetes mellitus	5	6.5
Cardiac diseases[a]	3	3.9
Asthma	3	3.9
Obstetric complications		
Pregnancy-induced hypertension	6	7.8
Oligohydramnios	4	5.2
Abruptio placentae	2	2.6
Breech presentation	2	2.6
Placenta previa	2	2.6

[a] Two cases of asymptomatic VSD and one case of mitral stenosis functional class I–II

Table 2 Fetal outcomes

Complications	Number	Percent
Low birth weight	34	44.1
Preterm birth	16	20.8
28–36 weeks	15	19.5
20–27 weeks	1	1.3
Fetal growth restriction	21	27.3
Fetal anomalies[a]	2	2.6
Perinatal death	3	3.9

[a] Cardiac anomalies [1], hypospadias [1]

Table 3 A comparison of hemoglobin levels, number of transfusions and birth weight between the group of hemoglobin H and beta-thalassemia/Hb E

	Thalassemia type	N	Mean	SD	*P* value [*t* test (2-tailed)]
Hb at first visit	HbH disease	52	8.301	2.1612	0.013
	Beta/E disease	23	6.980	1.8654	
Hb before delivery	HbH disease	52	7.263	2.0581	0.491
	Beta/E disease	23	6.896	2.2675	
Number of transfusions	HbH disease	51	0.69	1.104	0.030
	Beta/E disease	23	1.39	1.588	
Birth Weight	HbH disease	52	2,577.31	589.387	0.230
	Beta/E disease	23	2,399.57	579.431	

Discussion

To date, though thalassemia syndrome is very prevalent in our population, little is known about the pregnancy performance in patients with this disorder. Based on few small studies [4, 10, 11], the pregnancy outcomes among these patients are inconclusive. Most series have suggested that thalassemia disease has adversely impacted pregnancy outcomes, whereas some demonstrated favorable obstetrical and fetal outcomes if close follow-up and intensive treatment are instituted [4, 10, 11, 14].

Thalassemia exhibits a wide clinical spectrum ranging from mild manifestations to the severe anemia, requiring regular blood transfusion. The series presented here, one of the largest studies consisting of 80 patients, indicates that thalassemia syndrome is likely to be associated with adverse pregnancies outcomes, especially low birth weight, intrauterine growth restriction and preterm delivery, though most patients were managed to keep hemoglobin level exceeding 7.0 gm/dl. These findings were consistent with those reported by Nassar and et al. [13] who found that fetal growth restriction complicating 72% of pregnancies with thalassemia intermedia.

Unlike other previous reports on pregnancies with thalassemia, this study specifically focuses on beta-thalassemia/HbE disease and HbH disease. However, theoretically, the effects of these diseases may be similar, since the adverse impact is likely to be related to severity of anemia rather than thalassemic type itself.

As expected, the distribution of thalassemia types in this study included HbH disease (65%) beta-thalassemia/HbE disease (28.8%) and other types (6.3%). Since beta-thalassemia/HbE disease is usually more anemic than HbH disease and more likely to need blood transfusions, and shorter life expectancy, there is less frequency in the number of women become pregnant. As seen in the results, rate of fetal adverse outcomes among the mothers with beta-thalassemia/Hb E disease is worser than that in mothers with Hb H disease, though some outcomes were not significantly different, probably due to small sample size. Other type of thalassemia syndrome is rare during pregnancy.

Transfusion therapy is not currently a routine treatment approach for patients with thalassemia syndrome. Initiating regular blood transfusions in such patients remains a hard decision because of the heterogeneity of the disease. Patients who would benefit from such a measure include those with delayed growth, recurrent infections, and hypersplenism [2]. The physiologic anemia of pregnancy may be aggravated in patients with thalassemia syndrome. Also, the greater demand for hemoglobin for normal growth and development of the fetus might necessitate initiating transfusion or increasing the number of transfusions in those already requiring it. Hb levels in patients with thalassemia syndrome gradually decrease in the first and second trimesters, to increase again in the third trimester [15]. The benefits of frequent transfusion to the mother and infant (i.e., prevention of growth restriction) should be weighed against its negative effects (antibody development). Administration of erythropoietin in combination with iron and folic acid has been previously proposed and if proven effective might be an alternative to blood transfusion in such patients [16, 17]. Theoretically, pregnancy may aggravate cardiac decompensation in the patient with anemia-induced cardiac compromise; however, none of the patients in this study had cardiac failure secondary to anemia. This may be due to an attempt to keep hemoglobins level above 7.0 gm/dl during pregnancy. Nevertheless, this study indicates that such intervention may not probably effectively prevent adverse pregnancy outcomes. Notably, all patients in our study did not receive desferoxamine or iron chelating agents.

Thalassemia has been associated with an increased incidence of obstetrical complications [12, 13]. Two studies, on the other hand, that included a total of nine pregnancies in patients did not report evidence of an increased risk for ante-, intra-, or post-partum complications [4, 11]. Our patients had a high incidence of FGR and fetuses with low birth weight. Although, the patients in these studies were managed with the same protocol to maintain hemoglobin level of greater than 7.0 g/dl, the adverse impact on the fetuses was still high. This may be due to the fact that several women did not attend antenatal clinic in early gestation and hemoglobin levels were not corrected in early gestation. Whether strict control of hemoglobin level will result in better outcomes is yet to be elucidated. Some authors tried to maintain Hb levels above 10 g/dl since chronic maternal anemia can lead to a state of hypoxia leading to fetal growth restriction and preterm birth [11, 18]. However, since fetal growth restriction complicates more than half of pregnancies with thalassemia syndrome, the need for close antenatal follow-up and frequent sonographic assessment of fetal growth cannot be overemphasized.

Though, many authors demonstrated a higher rate of cesarean delivery among patients with thalassemia syndrome [6, 12, 13], the incidence in our study was not different from that in normal pregnant women in our hospital and we believe that thalassemia is not associated with an increased risk for cesarean delivery. Some authors indicated that the pregnant women often had spleen enlargement, bone deformity, and growth retardation resulting in high occurrence of cephalopelvic disproportion, which increases the rate of cesarean delivery [4, 6, 11, 19]. However, average fetal weight is often less than that in normal pregnancy; therefore, cesarean section due to cephalopelvic disproportion were not increased in our study, though cesarean birth due to fetal distress related to fetal growth restriction may be theoretically somewhat increased.

As already known, women during pregnancy and postpartum are at a higher risk of thrombosis due to hypercoagulable state of pregnancy. Likewise, patients with thalassemia also have an increased risk of thrombosis [20–24]. Therefore, these patients may further increase the risks of thromboembolism. However, none of patients in this study had such complications, indicating that thrombotic embolism risk may be only minimal for pregnant women in the East with thalassemia. This may be due to racial factors, as already known that thrombosis is relatively rare among eastern population compared to the western. It will be critical to study the biologic risk factors, thrombosis risk, and prophylaxis in prospective clinical trials with precise data regarding phenotype, genotype, and other thrombosis risk factors.

The weakness of this study is that it was retrospective descriptive without control; therefore, we could not know exactly whether the rate of adverse outcomes were different from that of the baseline. However, there was a high rate of fetuses with low birth weight, preterm birth and growth restriction when compared with that in general population.

In conclusion, thalassemia syndrome, including β-thalassemia/HbE disease and HbH disease during pregnancy can present unique management challenges and requires close maternal and fetal surveillance. In spite of an attempt to keep hemoglobin levels above 7.0 g/dl, the incidence of fetal growth restriction and preterm birth has been relatively high, though maternal complications are rather not different from general. Care for such pregnancies should be multidisciplinary, incorporating a maternal–fetal medicine specialist, a genetic counselor, and a hematologist. Although our patients did not experience cardiac, hemodynamic, hepatic, or kidney deterioration during pregnancy, these complications can occur, which stresses the need for careful monitoring throughout pregnancy.

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CLINICAL ARTICLE

Outcomes of pregnancies affected by hemoglobin H disease

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ABSTRACT

Objective: To determine the outcomes of pregnancies affected by hemoglobin H (HbH) disease. *Methods:* A retrospective cohort study was conducted with 120 women with singleton pregnancies complicated by HbH disease only. The controls-to-cases ratio was 2:1. *Results:* Maternal outcomes were similar in the 2 groups. The incidences of fetal growth restriction (relative risk [RR], 2.4; 95% confidence interval [CI], 1.60–3.50), preterm birth (RR, 1.4; 95% CI, 1.03–1.96), and low birth weight (RR, 1.9; 95% CI, 1.46–2.56) were significantly higher in the study than in the control group. The perinatal mortality rate was slightly higher in the study group. *Conclusion:* In spite of attempts to keep hemoglobin levels sufficiently high (>7.0 g/dL), pregnancies with HbH disease were significantly associated with increased risks of fetal growth restriction, preterm birth, and low birth weight.

1. Introduction

Adult hemoglobin (hemoglobin α2β2, or HbA) consists of two α- and two β-globin chains, each containing a heme group. The *α-globin* gene cluster, 5′-ζ-α2-α1-3′, is mapped to chromosome 16pter-p13.3. A person usually has four *α-globin* genes, and the normal genotype for diploid cells can be expressed as αα/αα. Four clinical syndromes resulting from the impaired synthesis of α-globin chains have been described. For each syndrome, a close correlation has been established between clinical severity and degree of synthesis impairment. There are 2 main groups of α-thalassemia determinants, α-thalassemia 1, which is characterized by the deletion of both loci from one chromosome (--/αα, also known as the Southeast Asian [SEA] type), and α-thalassemia 2, which is characterized by the loss of a single locus from one chromosome (-α/αα). Among our obstetric population, the carrier rate for the SEA-type deletion is as high as 6.6% [1].

Patients with HbH disease have only one α-globin gene(--/-α), often caused by the deletion of the other three *α-globin* genes. This group of disorders is sometime known as deletional HbH disease, because HbH (or β4 hemoglobin) is formed from the excess in β chains relative to the α chains. In Southeast Asia, the deletion of two *α-globin* genes, plus the inactivation of the third *α-globin* gene by a nondeletional mutation (such as Hb Constant Spring or Quong Sze mutations) are present in approximately 20% of patients with HbH disease [2]. Besides having the characteristics of thalassemia, individuals with HbH disease have moderately severe hemolytic anemia. Although red blood cell production is less ineffective in HbH disease than in other forms of thalassemia, HbH is highly unstable, resulting in an increased destruction of circulating red blood cells [3]. The disease is not always benign, especially during the neonatal period, in infancy, and during pregnancy [4], and it has been associated with hydrops fetalis [5,6]. In addition, anemia can necessitate transfusions and result in age-dependent iron overload (not necessarily related to the transfusions), hepatosplenomegaly, and osteopenia [7]. The personal and public health burdens of these syndromes have been largely overlooked.

None of the few published studies [8,9] on the outcomes of pregnancies complicated by HbH disease are cohort or case-control studies. The objective of the present study was to compare the maternal and fetal outcomes of pregnancies complicated by HbH disease with those of normal pregnancies.

2. Materials and methods

This retrospective controlled cohort study was conducted from January 1993 to December 2007. We used the obstetrics database of the Maternal-Fetal Medicine unit of the Department of Obstetrics and Gynecology of Chiang Mai University, patients' records, and a database concerning pregnancies with HbH disease. This particular database has been prospectively built since 1992 at our Maternal-Fetal Medicine unit, with new data entered on the day of each patient's discharge. The inclusion criteria for the study group were the following: (1) HbH disease diagnosed during or before pregnancy by hemoglobin typing or DNA analysis; (2) singleton pregnancy, with prenatal care and delivery at Maharaj Nakorn Chiang Mai hospital; (3) no other medical or surgical complications during pregnancy; and (4) available data for pregnancy outcome. The controls were recruited from the general obstetrics database, and the controls-to-patients ratio was 2:1. The inclusion criteria for controls were the following: (1) singleton pregnancy with no medical or surgical complication; (2) prenatal care and delivery at Maharaj Nakorn Chiang Mai hospital; (3) available data for pregnancy outcome; and (4) delivery on the same day, and as close in time as possible to the matched study patient. This study was

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conducted with the approval of the Research Ethics Committee of the Chiang Mai University Faculty of Medicine.

The pregnant women with HbH disease were usually followed up at the high-risk clinic by obstetricians and hematologists. They were screened for hepatitis B, HIV infection, and urinary tract infection; their hemoglobin concentration and hematocrit were checked regularly; and they were given echocardiograms as indicated. Generally, these patients were managed to maintain their hemoglobin levels higher than 7.0 g/dL.

Gestational age was established at the first visit, either by clinical estimation or by ultrasound. The patients were followed up for weight gain, blood pressure, fetal growth, and fetal heart rate. Hemoglobin levels were assessed every 2 to 3 weeks, and fetal growth was assessed by ultrasound in mid-pregnancy. Serial assessments were performed when fetal growth restriction was clinically suspected. Fetal outcomes of interest were the following: (1) perinatal death, defined as death in utero after 20 weeks of gestation or within 7 days of birth; (2) preterm birth, defined as a live birth before 37 weeks of gestation; (3) low birth weight, defined as a birth weight less than 2500 g; and (4) fetal growth restriction, defined as a birth weight less than the 10th percentile of the normal growth curve. In high-risk pregnancies, surveillance of viable fetuses was performed. Maternal records were reviewed for age, parity, hemoglobin concentration at the first visit and at delivery, number of blood transfusions, results of hemoglobin electrophoresis, history of splenomegaly, obstetric complications such as pre-eclampsia or postpartum hemorrhage, and mode of delivery. Neonatal records were reviewed for Apgar scores, fetal growth restriction, preterm birth, and low birth weight.

The data were analyzed using the SPSS, version 15.0 (SPSS, Chicago, IL, USA). Descriptive data are presented as number (percentage) or mean and standard deviation. Outcomes were compared between the study and control group using analysis of variance, the χ^2 test, or relative risks, as appropriate.

3. Results

There were 120 women in the study group and 240 in the control group. Nearly all women resided in the northern part of Thailand, mostly in Chiang Mai province (70.0% in the study group and 62.9% in the control group; $P=0.18$). Age, number of prenatal visits, place of residency, and parity were not significantly different in the 2 groups (more than half or the women in each group were nulliparous (56.7% in the study group and 57.5% in the control group; $P=0.88$) (Table 1). However, the percentage of women with a primary education or less was higher (35.8% vs 22.9%) and the mean hemoglobin level was significantly lower (8.19 g/dL vs 12.53 g/dL; $P<0.001$) in the study than in the control group. Of the 120 women in the study group, 13 (10.8%) had a prepregnancy history of splenectomy; and whereas only 12 (10%) had received blood transfusions before pregnancy, 58 (48%) needed blood transfusions during pregnancy (most received 1–5 transfusions).

Table 1
Baseline characteristics of pregnant women with HbH disease and controls [a]

Characteristic	Study group (n=120)	Control group (n=240)	*P* value
Maternal age, y	27.82±6.24	27.28±6.09	0.43 [b]
Parity			
Nulliparous	68	138	0.88 [c]
Parous	52	102	
Hb level, g/dL	8.19±2.21	12.53±2.05	0.000 [b]
No. of prenatal visits	8.62±3.83	8.67±3.61	0.91 [b]
Residency in Chiang Mai Province	84 (70.0)	151 (62.9)	0.183 [c]
Primary education or less	43 (35.8)	55 (22.9)	0.009 [c]

[a] Values are given as mean±SD, number, or number (percentage) unless otherwise indicated.
[b] By the *t* test.
[c] By the χ^2 test.

Table 2
Pregnancy outcomes in both groups [a]

Outcome	Study group (n=120)	Control group (n=240)	Relative risk (95% confidence interval)
Pre-eclampsia	11 (9.2)	11 (4.6)	1.36 (0.89–2.07)
Antepartum hemorrhage	7 (5.8)	6 (2.5)	1.46 (0.81–2.6)
Postpartum hemorrhage	6 (5.0)	2 (0.8)	2.71 (0.81–9.00)
Cesarean delivery	35 (29.2)	53 (22.1)	1.27 (0.93–1.74)
Fetal growth restriction	40 (33.3%)	18 (7.5)	2.37 (1.604–3.497)
Preterm birth	29 (24.2)	37 (15.4)	1.42 (1.03-1.96)
Low birth weight	54 (45.0)	53 (22.1)	1.94 (1.46–2.56)
Apgar score <7 at 5 minutes	7 (5.8)	11 (4.6)	1.18 (0.65–2.14)
Perinatal death	5 (4.2)	3 (1.3)	1.91 (1.1–3.34)
Fetal anomaly [b]	6 (5.0)	13 (5.4)	0.97 (0.71–1.33)

[a] Values are given as number (percentage) unless otherwise indicated.
[b] Anomalies included but were not restricted to cystic hygroma, gastroschisis, ventricular septal defects, ambiguous genitalia, ear abnormalities, digit deformities, club foot, and cleft lip.

The mean gestational age at birth was slightly less in the study group than that in the control group, but the difference was significantly different (37.16±2.83 weeks vs 37.77±2.45 weeks; $P=0.04$). The differences in the rates of maternal complications (which included pre-eclampsia, antepartum hemorrhage, postpartum hemorrhage, preterm rupture of membranes, and polyhydramnios), and the difference in the cesarean delivery rates were not significantly different in the 2 groups (Table 2). And whereas the rates of low Apgar scores (≤6) and of fetal anomalies were not significantly different, the rates of fetal adverse outcomes (which included preterm birth, low birth weight, and fetal growth restriction) were significantly higher in the study group (Table 2). Although there were few perinatal deaths, the perinatal mortality rate was significantly higher in the study group than that in the control group.

Because there were more poorly educated women in the study group, multivariate analysis was performed to identify whether this factor had a confounding effect. The results showed that only the type of thalassemia affected fetal outcomes.

4. Discussion

Most of the previous studies on pregnancies complicated by thalassemia are descriptive studies or cases series without controls, and most include different types of thalassemia. Because the available data have been scarce, relations between HbH disease and pregnancy outcomes have been inconclusive. Ong et al. [8] reported that HbH disease probably had no adverse effect on pregnancy, but Tantiweerawong et al. [9] found that HbH disease may adversely affect maternal and fetal health, causing low birth weight in particular.

The hemoglobin levels of women with HbH disease are typically between 8 and 9 gm/dL, but pregnancy can aggravate the severity of anemia. The mean hemoglobin level was 8.1 g/dL in our study, but the percentage of patients who needed blood transfusions to maintain their hemoglobin levels higher than 7.0 g/dL increased markedly during pregnancy, from 10% to nearly 50% (however, no patient needed splenectomy). Physiologic changes can worsen the course of HbH disease during pregnancy in other ways as well. Both anemia and pregnancy increase the heart load, and can place patients at risk for cardiac decompensation. However, this serious complication did not occur in our study, probably owing to interventions such as blood transfusions.

Unlike the small case series published by Ong et al. [8], our controlled cohort study highlights a link between HbH disease and adverse pregnancy outcomes. It indicates that HbH disease places fetuses at significant risk for growth restriction, preterm birth, and low birth weight, resulting in increased perinatal mortality. Although we attempted to keep out patients' hemoglobin levels higher than 7.0 g/dL, adverse fetal outcomes were major problems. This

suggests that blood transfusions may not effectively prevent adverse fetal outcomes, and that a new guideline for the management of pregnancies affected with HbH disease is needed.

Common obstetric complications, such pre-eclampsia, antepartum hemorrhage, and postpartum hemorrhage, were not significantly associated with HbH disease in our study. Some authors reported a higher cesarean delivery rate among women with β-thalassemia, and suggested the increase to be due to the splenomegaly, pelvic bone deformity, and abnormal pelvic configuration often found in these women [10–13]. The cesarean delivery rate was not significant associated with HbH disease in this study with women who had α-thalassemia, and the greater number of small fetuses in the study group may have contributed to a greater chance of vaginal delivery.

Pregnancy may aggravate anemia-related cardiac decompensation, but none of our patients had cardiac failure secondary to anemia. Although the constant demand for hemoglobin from developing fetuses may necessitate initiating maternal transfusions, or increasing their frequency in women already requiring them, the benefits of frequent transfusions (eg, the prevention of growth restriction) should be weighed against their drawbacks (eg, antibody development)—keeping in mind that it is not known whether a strict control of hemoglobin levels results in better outcomes. Since chronic maternal anemia can lead to a state of hypoxia, itself leading to fetal growth restriction and preterm birth, some authors have tried to maintain hemoglobin levels higher than 10 g/dL [14]. However, since fetal growth restriction complicates only about one-third of pregnancies with HbH disease, serial growth assessment should be recommended first. It could be suggested that, in this study, some cases of fetal growth restriction may have been be linked to poor prenatal attendance by women of low education, or a lack of proper surveillance in late pregnancy, or a lack of correction of hemoglobin levels in early pregnancy. However, it is unlikely that lower educational levels were responsible for fetal growth restriction because the number of prenatal care visits was not significantly different in the control group, and because multivariate analysis showed that a poor education had no significant impact on fetal outcome.

Medications containing erythropoietin or iron-chelating agents are not known to be safe or effective during pregnancy. To our best knowledge, such medications should not be used as alternatives to blood transfusion in pregnant women with HbH disease, and should be avoided altogether during pregnancy. None of our patients received any such agents.

Some limitations of this study should be mentioned. Because we performed blood transfusions in several cases, we could not truly assess the natural effect of HbH disease on pregnancy. Additionally, we did not divide patients according to disease subtype. Although nearly all patients had the deletion type of HbH, some had HbH Constant Spring, a nondeletion type.

Since both pregnancy and thalassemia are associated with a higher risk of thrombosis due to a hypercoagulable state [1,15–19], pregnant women with HbH disease will theoretically be at higher risk for thromboembolism. None of the patients in this study had this complication, which may indicate that, in our population, this risk is only minimal for pregnant women with HbH disease (thrombosis is known to be relatively rare among eastern populations).

In conclusion, this study indicates that, in spite of an attempt to keep hemoglobin levels above 7.0 g/dL, pregnancies with HbH disease were significantly associated with increased risks of fetal growth restriction, preterm birth, low birth weight, and perinatal mortality, whereas the numbers of maternal complications were not significant increased.

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CLINICAL ARTICLE

Outcomes of pregnancies complicated by beta-thalassemia/hemoglobin E disease

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ABSTRACT

Objective: To assess the outcomes of pregnancies affected by beta-thalassemia/hemoglobin E (β-thal/HbE) disease. *Methods:* A retrospective cohort study was conducted with 54 women with singleton pregnancies complicated by β-thal/HbE disease only. The controls-to-cases ratio was 2:1. *Results:* Although maternal outcomes were similar in both groups, gestational age at birth and birth weight were significantly lower in the study group and the cesarean delivery rate was significantly higher in that group (relative risk [RR], 2.1). The incidences of fetal growth restriction, preterm birth, and low birth weight were also significantly higher in the study group, with RRs of 2.8, 2.7, and 5.6, respectively. *Conclusion:* Pregnancies affected by β-thal/HbE disease were significantly associated with an increased risk of fetal growth restriction, preterm birth, and low birth weight.

1. Introduction

One of the most common hemoglobin variants worldwide, hemoglobin (Hb) E results from a single beta-chain substitution of glutamic acid for lysine at codon 26. The heterozygous E trait is common in Southeast Asia and usually asymptomatic. On the other hand, the doubly heterozygous beta-thalassemia/hemoglobin E (β-thal/HbE) disease is a major public health problem in Southeast Asia, a common cause of childhood anemia, and a cause of severe anemia requiring transfusions during pregnancy. These 2 genetic defects in patients with β-thal/HbE disease are different mutations of *β-globin* genes located on chromosome 11. The clinical manifestations of β-thal/HbE disease are heterogeneous. Although many patients have a milder form of the illness, with a virtually asymptomatic early childhood, the severe end of the spectrum is characterized by profound anemia necessitating medical attention in the first year of life, then regular transfusions and sometimes splenectomy.

Expensive and risky treatments such as hypertransfusion, regular iron chelation, and bone marrow transplantation can be considered for selected patients, but conventional treatment with regular transfusions and iron chelation is not possible for most patients in low-income countries. Cure is still limited to the small number of patients who have undergone bone marrow or stem cell transplantation. The patients are usually anemic, with hemoglobin levels between 5 and 9 g/dL and at least some splenomegaly [1,2]. Until recently, women with β-thal/HbE disease rarely became pregnant but a more focused medical care has caused their fertility to increase. The physiologic changes during pregnancy, however, further heighten the severity of anemia in the mother, which could affect both clinical symptoms and pregnancy outcome [3]. Since low maternal hemoglobin levels can lead to fetal hypoxia, pregnancies affected by β-thal/HbE disease may be associated with a high rate of obstetric complications, especially fetal growth restriction and preterm labor [4,5].

There are some reports on pregnancy outcomes among women with thalassemia [6–9], but no published studies—whether case series or cohort study—specifically focusing on pregnancy complicated by β-thal/HbE disease. The objective of the present study was to compare the maternal and fetal outcomes of pregnancies complicated by β-thal/HbE disease with those of normal pregnancies.

2. Materials and methods

This retrospective controlled cohort study was conducted at the Maternal-Fetal Medicine unit of the Department of Obstetrics and Gynecology of Chiang Mai University using a database concerning pregnancies affected with β-thal/HbE disease. This particular database has been prospectively built since 1992 at our Maternal-Fetal Medicine unit, with new data entered on the day of each patient's discharge. The inclusion criteria for the study group were the following: (1) β-thal/HbE disease diagnosed during or before pregnancy by hemoglobin typing; (2) singleton pregnancy, with prenatal care and delivery at Maharaj Nakorn Chiang Mai hospital; (3) no other medical or surgical complications during pregnancy; and (4) available data for pregnancy outcome. The controls were recruited from the general obstetrics database, and the controls-to-patients ratio was 2:1. The inclusion criteria for controls were the following: (1) singleton pregnancy with no medical or surgical complication; (2) prenatal care and delivery at Maharaj Nakorn Chiang Mai hospital; (3) available data for pregnancy outcome; and (4) delivery on the same day, and as close in time as possible to that of the matched study patient. This study was conducted with the approval of the Research Ethics Committee of the Chiang Mai University Faculty of Medicine.

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Most of the pregnant women with β-thal/HbE disease were regularly followed up at the high-risk clinic by obstetricians and hematologists. They were screened for hepatitis B, HIV infection, and urinary tract infection; their hemoglobin concentration and hematocrit were checked regularly; their liver function was evaluated and they received echocardiograms. Generally, these patients were managed to maintain their hemoglobin levels higher than 7.0 g/dL.

Gestational age was established at the first visit, either by clinical estimation or by ultrasound. The patients were followed up for weight gain, blood pressure, fetal growth, and fetal heart rate. Hemoglobin levels were assessed every 2 to 3 weeks, and fetal growth was assessed by ultrasound in mid-pregnancy. Serial assessments were performed when fetal growth restriction was clinically suspected. Fetal outcomes of interest were the following: (1) perinatal death, defined as death in utero after 20 weeks of gestation or within 7 days of birth; (2) preterm birth, defined as a live birth before 37 weeks of gestation; (3) low birth weight, defined as a birth weight less than 2500 g; and (4) fetal growth restriction, defined as a birth weight less than the 10th percentile of the normal growth curve. In high-risk pregnancies, surveillance of viable fetuses was performed. Maternal records were reviewed for age, parity, hemoglobin concentration at the first visit and at delivery, number of blood transfusions, results of hemoglobin electrophoresis, history of splenomegaly, obstetric complications such as pre-eclampsia or postpartum hemorrhage, and mode of delivery. Neonatal records were reviewed for Apgar scores, fetal growth restriction, preterm birth, and low birth weight.

The data were analyzed using SPSS version 15.0 (SPSS, Chicago, IL, USA). Descriptive data are presented as number (percentage) or mean and standard deviation. Outcomes were compared between the study and control group using analysis of variance, the χ^2 test, or relative risks, as appropriate.

3. Results

There were 54 women in the study group and 108 in the control group. Nearly all women resided in the northern part of Thailand, mostly in Chiang Mai province (59.3% in the study group and 63.9% in the control group; $P=0.57$). Age, number of prenatal visits, place of residency, level of education, and parity were not significantly different in the 2 groups (the percentage of nulliparous women was higher in the β-thal/HbE group, but the difference was not significant [68.5% vs 58.0%; $P=0.21$]) (Table 1). However, the mean hemoglobin levels were significantly lower in the β-thal/HbE group than in the control group (8.12 g/dL vs 12.76 g/dL; $P<0.001$) (Table 1). In the study group, 8 patients had undergone splenectomy before pregnancy. In that group, 19 patients (35%) did not need blood transfusions before pregnancy but most of them required 1 to 5 transfusions during pregnancy (mean ±SD, 2.40 ± 1.42).

Gestational age differed significantly in the study and control groups (36.37 ± 2.97 weeks vs 38.15 ± 3.14 weeks; $P=0.001$). The differences between the 2 groups in the rates of maternal complications (ie, pre-eclampsia, antepartum hemorrhage, postpartum hemorrhage, preterm rupture of membranes, polyhydramnios) and the cesarean delivery rates were not statistically significant (Table 2). When the numbers of cesarean deliveries were compared between the 2 groups by indication (ie, fetal distress, failure of labor to progress, or other), the number in each group was too low to show a significant difference ($P>0.05$).

And whereas the rates of low Apgar scores (≤6), perinatal mortality, and fetal anomalies were not significantly different in the 2 groups, the rates of fetal adverse outcomes (which included preterm birth, low birth weight, and fetal growth restriction) were significantly higher in the study group (Table 2).

Table 1
Baseline characteristics of pregnant women with β-thal/HbE disease and controls [a]

Characteristic	Study group (n=54)	Control group (n=108)	*P* value
Maternal age, y	27.09±6.28	26.67±4.43	0.62 [b]
Parity			
Nulliparous	37	63	0.21[c]
Parous	17	45	
Hb level, g/dL	8.13±2.76	12.76±9.35	0.000[b]
No. of prenatal visits	9.33±3.96	9.88±2.82	0.08[b]
Residency in Chiang Mai Province	32 (59.3)	69 (3.9)	0.57[c]
Primary education or less	22 (40.7)	35 (32.4)	0.30[c]

[a] Values are given as mean±SD, number, or number (percentage) unless otherwise indicated.
[b] By the *t* test.
[c] By the χ^2 test.

Table 2
Pregnancy outcomes in both groups[a]

Outcome	Study group (n=54)	Control group (n=108)	Relative risk (95% confidence interval)
Pre-eclampsia	3 (5.6)	7 (6.5)	0.95 (0.62–1.45)
Antepartum hemorrhage	3 (5.6)	6 (5.6)	1.00 (0.62–1.61)
Postpartum hemorrhage	3 (5.6)	1 (0.9)	2.71 (0.49–14.84)
Cesarean delivery	15 (27.8)	10 (9.3)	2.11 (1.39–3.19)
Fetal growth restriction	24 (44.4)	9 of 99 (8.3)	2.81 (1.60–4.95)
Preterm birth	19 (35.2)	8 (7.4)	2.71 (1.86–3.95)
Low birth weight	38 (70.4)	10 (9.3)	5.64 (3.50–9.09)
Apgar score <7 at 5 minutes	1 (1.4)	4 (3.7)	0.83 (0.53–1.30)
Perinatal death	1/53 (1.9)	2/106 (1.9)	1.00 (0.45–2.24)
Fetal anomaly	2/52 (3.7)[b]	4 (3.7)[c]	1.00 (0.56–1.78)

[a] Values are given as number (percentage) within the entire group unless otherwise indicated.
[b] The anomalies concerned the genitalia (ambiguous) and the ears.
[c] There was 1 case each of anencephaly, cystic hygroma, club foot, and cleft lip.

4. Discussion

To the best our knowledge, this is the first controlled cohort study of the effects of β-thal/HbE disease on pregnancy outcomes. Although there have been several studies on pregnancies complicated by thalassemia, most were descriptive studies or cases series without controls and most included patients with mixed types of thalassemia.

The severity of β-thal/HbE disease depends on several factors, especially gene mutations, and the clinical symptoms of affected patients can be remarkably diverse. A limitation of this study is that neither genotype nor mutations were determined. But considering that patients with severe disease very rarely become pregnant, and that if they do, they usually need more transfusions than we administered, it is likely that most of our patients had mild forms of the disease. Whereas β-thal/HbE disease is the most common form of severe thalassemia in our general population, with an incidence as high as 1% in northern Thailand, only 0.2% of all pregnant women were affected by β-thal/HbE disease in our study. Transfusion therapy is not routinely used in patients with the disease at our hospital. Because of the heterogeneity of the disease, initiating regular blood transfusions remains a problematic decision. Patients who would benefit from such a measure include those with recurrent infections, hypersplenism, and/or signs of delayed growth [1].

Our results highlight β-thal/HbE disease as an indicator for possible adverse pregnancy outcomes. Unlike Scanlon et al. [10], who report no association between maternal anemia and small-for-gestational age fetuses, we found β-thal/HbE disease to be associated with fetal growth restriction (RR, 2.81; 95% CI, 1.60–4.95) as well as preterm birth and low birth weight. Although we tried to keep the patients' hemoglobin levels higher than 7.0 gm/dL, adverse fetal outcomes were the major problems in our study. This suggests that blood transfusions may not effectively prevent adverse fetal outcomes, and that a new guideline for the management of pregnancies affected with β-thal/HbE disease is needed [11,12]. Moreover, increasing the number of blood transfusions can be detrimental to patients, especially during pregnancy, a period of

hypervolemia. Pregnancy may aggravate anemia-related cardiac decompensation, but none our patients had cardiac failure secondary to anemia. Although the constant demand for hemoglobin from developing fetuses may necessitate initiating maternal transfusions, or increasing their frequency in women already requiring them, the benefits of frequent transfusions (eg, the prevention of growth restriction) should be weighed against their drawbacks (eg, antibody development)—keeping in mind that it is not known whether a strict control of hemoglobin levels results in better outcomes. Since chronic maternal anemia can lead to a state of hypoxia, itself leading to fetal growth restriction and preterm birth, some authors have tried to maintain hemoglobin levels higher than 10 g/dL [7,13]. However, since fetal growth restriction complicates only about one-third of pregnancies with HbH disease, serial growth assessment should be recommended first. Additionally, little is known regarding other treatment modalities for β-thal/HbE disease during pregnancy. Administration of erythropoietin in combination with iron and folic acid has been proposed, and if proven effective, this treatment could become be an alternative to blood transfusion. The safety of desferoxamine or iron chelating agents during pregnancy is not known. None of our patients received any such agents.

It could be suggested that some cases of fetal growth restriction may be linked to poor prenatal attendance, or a lack of proper surveillance in late pregnancy, or a lack of correction of hemoglobin levels in early pregnancy. This is unlikely, however, because the number of prenatal care visits was not significantly different in the control group.

Whether β-thal/HbE disease increases maternal problems is still controversial. Some authors found a relationship between the disease and an increased incidence of complications before, during, and after delivery [9], whereas some did not [7,8], and differences in quality of care during pregnancy may explain the differing reports. Although anemia places patients at risk for cardiac decompensation, we did not observe cardiac failure secondary to anemia or an increased incidence of other common obstetric complications among our patients.

The cesarean delivery rate was significantly higher for patients with β-thal/HbE disease despite a significantly lower birth weight in this group. The higher rate may be due to an association between fetal distress and fetal growth restriction, but the number of patients in any subgroup categorized by indications was too small to show a significant difference. On the other hand, the pelvic bone deformity often seen in these patients may lead to cephalopelvic disproportion and explain the higher rate of cesarean delivery among them [4,7,8,14].

Since both pregnancy itself and thalassemia are associated with a higher risk of thrombosis due to hypercoagulable state [15–17], pregnant women with β-thal/HbE disease are at higher risk for thromboembolism, especially in the early post partum. None of the patients in this study had this complication, which may indicate that, in our population, this risk is only minimal for pregnant women with β-thal/HbE disease (thrombosis is known to be relatively rare among eastern populations). Our findings therefore do not support prophylaxis for thromboembolism in pregnancies complicated by β-thal/HbE disease in our region.

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High Fetal Splenic Artery Peak Velocity in Fetuses With Hemoglobin Bart Disease

A Preliminary Study

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Objective. The purpose of this study was to evaluate the role of the splenic artery (SPA) peak systolic velocity (PSV) in identifying fetuses with hemoglobin (Hb) Bart disease among pregnancies at risk for the disease. ***Methods.*** Pregnancies at risk for fetal Hb Bart disease scheduled for cordocentesis at 18 to 25 weeks' gestation at Maharaj Nakorn Chiang Mai Hospital were recruited into the study. The SPA PSV was measured before cordocentesis, and the final fetal diagnosis of Hb Bart disease was based on fetal Hb typing using high-performance liquid chromatography. ***Results.*** Seventy-six singleton pregnancies at risk for fetal Hb Bart disease were sonographically evaluated for the SPA PSV and underwent cordocentesis for fetal blood analysis. Among the 76 recruited pregnancies, 17 fetuses with Hb Bart disease were finally diagnosed by fetal blood analysis with high-performance liquid chromatography, and the remainder had no abnormalities or had the α-thalassemia 1 trait and were defined as unaffected fetuses. The mean SPA PSVs ± SD for the unaffected and affected fetuses were significantly different: 21.17 ± 3.7 cm/s (range, 13.8–29.9 cm/s) and 26.12 ± 3.6 cm/s (range, 20.4–31.5 cm/s) respectively. The SPA PSV of the affected fetuses was higher than that of the unaffected ones (Wilcoxon signed rank test, $P < .001$). ***Conclusions.*** Splenic artery PSV assessment at mid pregnancy may have a potential role in identifying fetuses with Hb Bart disease. Further studies to evaluate the effectiveness of the SPA PSV in differentiating affected from unaffected fetuses among pregnancies at risk are desirable. ***Key words:*** Doppler sonography; hemoglobin Bart disease; splenic artery peak systolic velocity.

Abbreviations
Hb, hemoglobin; PSV, peak systolic velocity; SPA, splenic artery

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Among fetuses with hemoglobin (Hb) Bart disease, who are inevitably either stillborn or dead soon after birth, early pregnancy termination before the development of hydrops can prevent serious obstetric complications.[1] These include preeclampsia, dystocia, postpartum hemorrhage due to a large placenta, and the psychological burden of carrying a nonviable fetus to term. Therefore, the ability to make a prenatal diagnosis and to perform early termination of the pregnancy is essential. Over the last 2 decades, researchers have attempted to diagnose fetal anemia using sonographic criteria in fetuses at risk for immune hydrops. The concept is appealing, but the results have

been varied. Traditionally, clinicians have relied on invasive methods to detect fetal anemia either indirectly by measurement of amniotic fluid bilirubin levels or more recently by direct measurement of fetal hematocrit levels through cordocentesis. Both methods pose a risk to the fetus. Fetal loss rates associated with amniocentesis and cordocentesis are 0.5% to 1%[2,3] and 1.4% to 2.7%,[4,5] respectively. As a screening tool, sonographic assessment could prevent these complications by decreasing the number of invasive procedures required in the management of fetal anemia. To be useful, sonographic findings must reliably detect fetal anemia before the onset of overt hydrops fetalis. The fetus can compensate for moderate degrees of anemia by hemodynamic adjustments, but once the Hb deficit exceeds 7 g/dL, the fetal functional reserve is exhausted, with resulting hydropic changes.[6]

There is ample evidence showing that fetal anemia, especially secondary to maternal alloimmunization, is highly associated with several alterations in fetal hemodynamics, including the middle cerebral artery and splenic artery (SPA) circulation, which could be assessed by Doppler sonography.[7,8] Theoretically, assessment of the SPA peak systolic velocity (PSV) may predict or identify a fetus with Hb Bart disease among fetuses at risk. Assessments of the SPA PSV in early studies were shown to be useful in predicting fetal anemia secondary to maternal alloimmunization.[7] Likewise, they may also be useful in differentiating affected fetuses from unaffected ones among fetuses at risk for Hb Bart disease. We postulate that the SPA PSV could differentiate fetuses with Hb Bart disease from unaffected ones. The objective of this study was to evaluate the role of the SPA PSV in identifying fetuses with Hb Bart disease among pregnancies at risk for the disease.

Materials and Methods

This study was conducted with approval of the Research Ethical Committee at a tertiary center, Maharaj Nakorn Chiang Mai Hospital, where the program of prenatal control for severe thalassemia syndrome has been well established.[9] Between July 2006 and March 2008, pregnant women at risk of having fetuses with Hb Bart disease and attending the antenatal care clinic were recruited into the study with written informed consent. All patients underwent sonographic examinations by the authors, including SPA PSV assessment before cordocentesis. Inclusion criteria were as follows: (1) singleton pregnancies at a gestational age of 18 to 25 weeks, determined by accurate last menstrual period or fetal sonographic parameters in the first half of pregnancy, and (2) couples at risk of having fetuses with Hb Bart disease, both having carrier status confirmed by polymerase chain reaction (**Southeast Asian deletion** type). The final diagnosis of Hb Bart or non-Hb Bart disease was based on cordocentesis and fetal blood analysis using high-performance liquid chromatography. Exclusion criteria were as follows: (1) fetal anomaly or anemia other than hydrops fetalis secondary to Hb Bart disease and (2) loss to follow-up or final diagnosis could not be obtained.

All sonographic examinations were performed with real-time ultrasound equipment (SSD-Alpha 10; Aloka Co, Ltd, Tokyo, Japan) and transabdominal 3.5- to 5-MHz curvilinear transducers. On examination, the pregnant women were in a semirecumbent position. All measurements were performed in a period of no fetal breathing and movement. Measurement of SPA PSV Doppler waveforms was done as follows: (1) the fetal abdomen was first imaged in an axial view at the level of the stomach by means of real-time sonography with a 3.5- or 5-MHz transducer, as appropriate, with color and pulsed Doppler capabilities; (2) color Doppler imaging was used to localize the SPA as it arose from the celiac axis and coursed behind the stomach into the splenic hilum (Figure 1); (3) pulsed Doppler evaluation was performed with a sample volume of 2 mm, a pass filter between 0 and 50 Hz, and an angle of insonation between 0° and 30° from the ultrasound beam, with angle correction to obtain the best waveforms; (4) the sample volume was positioned along the SPA in close proximity to its origin from the celiac axis; (5) the PSV and lowest diastolic velocity were calculated with electronic calipers; (6) 3 consecutive waveforms were analyzed, and the results were averaged; (7) measurements were obtained during fetal apnea; and (8) the above steps were repeated at least 3 times.

The main outcome of the measurement was a comparison of the mean SPA PSV between affected fetuses and unaffected ones among fetuses at risk at 18 to 25 weeks' gestation. The data were analyzed with SPSS version 15.0 software (SPSS Inc, Chicago, IL). Descriptive data are presented as mean ± SD. The comparison between both groups was analyzed by the Wilcoxon signed rank test.

Results

During the study period (July 2006 to March 2008), 76 singleton pregnancies at risk for Hb Bart disease were sonographically evaluated for the SPA PSV and underwent cordocentesis for fetal blood analysis at 18 to 25 weeks' gestation. Of the 76 recruited pregnancies, the mean maternal age was 30.21 ± 5.27 years (range, 18–44 years), and the mean gestational age was 20.66 ± 1.98 weeks (range, 18-25 weeks). Seventeen fetuses with Hb Bart disease had a final diagnosis by fetal blood analysis with high-performance liquid chromatography, and the remainder had no abnormalities or had the α-thalassemia 1 trait, defined as unaffected fetuses.

The mean SPA PSVs of the unaffected fetuses and fetuses with Hb Bart disease were 21.17 ± 3.7 cm/s (range, 13.8–29.9 cm/s) and 26.12 ± 3.6 cm/s (range, 20.4–31.5 cm/s), respectively, whereas the median values were 21.1 cm/s (95th percentile, 27.5 cm/s) and 27.2 cm/s (95th percentile, 31.5 cm/s), respectively. The mean SPA PSV of affected fetuses was higher than that of unaffected ones at all gestational weeks, as shown in Table 1 and the scatterplot in Figure 2. The median SPA PSV of all fetuses was 21.95 cm/s (95th percentile, 30 cm/s), and the difference in the SPA PSVs between both groups was significantly different (Wilcoxon signed rank test, $P < .001$).

Of the 17 fetuses with Hb Bart disease, nearly half of them (8) had some sonographic signs of early hydrops fetalis: at least 2 areas of fluid collections such as pleural effusions, ascites, or subcutaneous edemas. All of these hydropic fetuses had a high SPA PSV that did not overlap that of the unaffected fetuses at each gestational week.

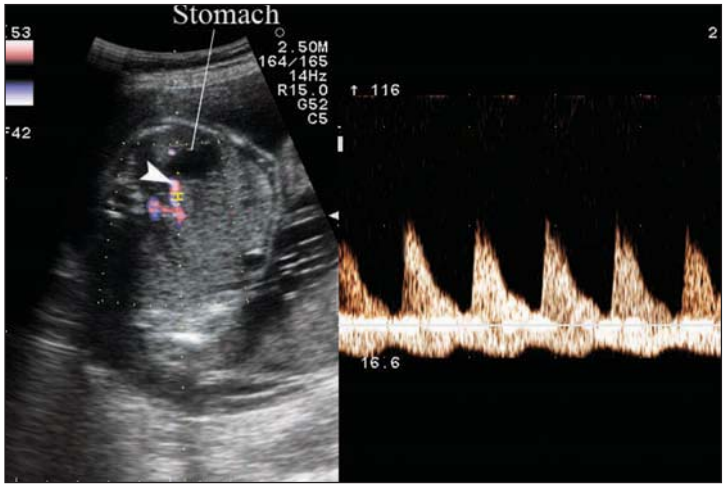

Figure 1. Color eFlow image of the SPA (arrowhead) located just behind the stomach and Doppler waveforms in a fetus with Hb Bart disease.

Discussion

Because the spleen is the organ directly responsible for both red blood cell destruction and production, changes in SPA circulation, as assessed by Doppler velocimetry, are likely to correlate with the degree of fetal anemia. An increase in the dimensions of the spleen in severe fetal anemia has also been noted.[10] On the basis of previous reports, there was a significant correlation between an increasing SPA PSV and the Hb deficit in fetuses with Rh isoimmunization.[7] Therefore, theoretically, the SPA PSV may possibly have a potential role in predicting affected fetuses among those at risk for Hb Bart disease, in whom anemia usually develops as early as the beginning of the second trimester, although hydropic changes usually occur in the second

Table 1. Comparison of Mean SPA PSV Between Unaffected and Affected Fetuses by Gestational Age

Gestational Age, wk	Unaffected Fetuses		Affected Fetuses	
	n	Mean PSV, cm/s	n	Mean PSV, cm/s
18	8	16.48	2	21.65
19	13	19.07	4	22.7
20	9	20.39	2	25.7
21	11	22.44	4	28.78
22	7	23.09	2	27.9
23	5	25.38	1	28
24	3	25.27	1	28.4
25	3	24.87	1	31.3
Total	59	21.17	17	26.12

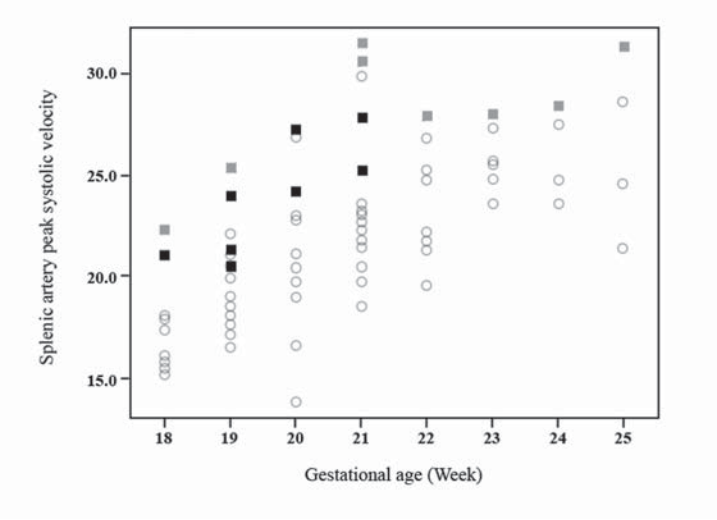

Figure 2. Scatterplot for the SPA PSV of unaffected fetuses (circles) and fetuses with Hb Bart disease (black squares, without hydropic changes; gray squares, with hydropic changes) at each gestational age.

half of pregnancies. Fetuses with Hb Bart disease are associated with a hyperdynamic circulation and low blood viscosity secondary to anemia, resulting in an elevated PSV in several vessels, as we observed in the middle cerebral artery.[11] We postulated that such a hemodynamic change should also be found in the splenic circulation. Despite its apparent importance, little research has been devoted to the spleen.[10,12,13]

The results of this study confirmed our hypothesis. There was a significant correlation between a high SPA PSV and Hb Bart disease. This finding is likely to be of clinical importance because SPA PSV assessment could distinguish affected fetuses from unaffected ones, although the differentiation was not perfect because of some overlapping values among affected and unaffected fetuses. The SPA PSV seems to be useful in identifying appropriate candidates for cordocentesis. Because fetuses with Hb Bart disease always have severe anemia, the Doppler index should be able to distinguish affected from unaffected ones among fetuses at risk for the disease. The ability to predict severe anemia in fetuses may possibly be important because unless anemia is identified, the risk may be very low, and invasive diagnostic procedures can possibly be obviated, although serial sonography may still be required.

Notably, the SPA PSV values of most fetuses with Hb Bart disease in this series, even in cases with hydropic signs, were in the normal range on the basis of a nomogram reported by Bahado-Singh et al[7] (<1.5 multiples of the median), even though they were very high when compared with those of unaffected fetuses in our own series. This may signify that a cutoff value of 1.5 multiples of the median or a nomogram for a different population may not be appropriate for use in our population.

On the basis of this small series, a high SPA PSV among fetuses at risk for Hb Bart disease at mid pregnancy is highly suggestive, but not absolutely diagnostic, of an affected pregnancy, for which an invasive diagnostic procedure is strongly indicated. Women scheduled for cordocentesis as a prenatal diagnostic technique for fetal Hb Bart disease may benefit from SPA PSV assessment. Theoretically, it is possible that a normal SPA PSV at mid pregnancy is relatively reassuring and may obviate further invasive procedures to confirm normality, although follow-up sonography may be required. Because the main objective of prenatal diagnosis of Hb Bart disease is reduction of maternal morbidity rather than prevention of the birth of affected neonates, serial measurements of the SPA PSV may be alternative methods for predicting the disorder, particularly in areas where resources for prenatal diagnosis are limited. In our population, the main prenatal diagnostic technique is cordocentesis at 18 to 21 weeks, and it is performed only in tertiary centers. It is very important for community hospitals to refer only cases at high risk for Hb Bart disease for invasive prenatal diagnostic procedures. Therefore, measurement of the SPA PSV may be an alternative method for identifying cases for referral or follow-up with serial sonography in these hospitals. With this approach, invasive procedures can be performed selectively, and fewer fetuses will be lost unnecessarily. The reduction in medical expenses and procedure-related fetal loss is likely to be substantial. Although a few affected fetuses may be missed by this approach, these cases can be suspected or detected later by serial sonography. Moreover, because the disease is uniformly lethal, and termination of pregnancy can be offered any time when the

diagnosis is made reliably, missing it in a very small number of cases will not influence the overall outcome substantially.

Several limitations of this study should be mentioned. (1) This was only preliminary experience including only a small number of cases with early hydrops fetalis. Several affected fetuses had already had hydropic changes during examination. Among these fetuses, the SPA PSV may be of little clinical value because other hydropic signs warrant further workup without the need of SPA PSV measurement. (2) We did have our own nomogram for the SPA PSV. A nomogram for Western pregnancies may not have been appropriate for use in our study. As seen in the unaffected fetuses in this study, the SPA PSV was apparently lower than that in the nomogram of Bahado-Singh et al.[7] This may be due to racial factors or gestational age. Notably, the nomogram of Bahado-Singh et al[7] included fetuses after 20 weeks' gestation, whereas most fetuses in this study were confined to 20 weeks or less. (3) The sample size was too small to develop a receiver operating characteristic curve to determine the appropriate SPA PSV cutoff point for differentiating affected from unaffected fetuses. (4) We did not determine the concentration of fetal Hb; therefore, we could not correlate the degree of anemia and the SPA PSV. (5) Some bias in this study might have existed. This was due to the fact that Doppler evaluation of the SPA was not a blind method because the examiner knew the morphologic characteristics of the hydropic changes from conventional sonograms, especially in the cases in which hydrops fetalis had already occurred at the time of examination. Therefore, the diagnosis of Hb Bart disease or a high SPA PSV could have been anticipated.

We suggest that nomograms for the SPA PSV be developed for specific populations, especially in areas where Hb Bart disease is prevalent. Future studies should focus on fetuses in early pregnancy without hydropic signs to see whether the SPA PSV can differentiate affected from unaffected fetuses before sonographic signs of hydrops fetalis become evident.

In summary, this study suggests that SPA PSV assessment has a potential role in differentiating affected from unaffected fetuses among those who are at risk for Hb Bart disease, and this may be a new marker for helping us identify Hb Bart disease among fetuses at risk during early gestation and suggesting how to follow at-risk pregnancies appropriately. Additionally, in our experience, main SPA PSV measurements are relatively easy to obtain, although high-resolution sonography may be desirable. However, because this study was very preliminary, further studies are needed to determine the best cutoff values for differentiating affected from unaffected fetuses and to test for accuracy in large-scale populations. Moreover, future studies should focus only on fetuses at risk during early gestation, when sonographic signs of hydrops fetalis have not yet developed, and a nomogram for the SPA PSV at each gestational week in the first half of pregnancy should be developed for each specific population.

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Identification of fetuses with hemoglobin Bart's disease using middle cerebral artery peak systolic velocity

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KEYWORDS: Hb Bart's disease; homozygous α-thalassemia-1; MCA-PSV; middle cerebral artery peak systolic velocity

ABSTRACT

Objectives *To determine the sensitivity and specificity of middle cerebral artery peak systolic velocity (MCA-PSV) in mid-pregnancy for the identification of homozygous α-thalassemia-1 (hemoglobin (Hb) Bart's disease) in fetuses at risk of the disease.*

Methods *A total of 88 pregnancies (91 fetuses) at risk of Hb Bart's disease and undergoing MCA-PSV measurement before cordocentesis at 18–22 weeks of gestation were recruited into the study. Definitive diagnosis was made using the gold standard technique of Hb typing by high-performance liquid chromatography.*

Results *The mean ± SD age of the 88 pregnant women recruited into the study was 28.3 ± 5.7 years, the gestational age was 18.8 ± 1.1 weeks and the incidence of Hb Bart's disease was 22% (20 fetuses). Using MCA-PSV above 1.5 multiples of the median as a cut-off point, the sensitivity of MCA-PSV for detecting affected fetuses was 85% (17/20 cases), with a specificity of 100%, and positive and negative predictive values of 100% and 95.9% respectively. Three of 20 fetuses with Hb Bart's disease had normal MCA-PSV.*

Conclusions *MCA-PSV assessment in mid-pregnancy is a useful method for identifying Hb Bart's disease with high sensitivity and specificity among fetuses at risk, and may allow avoidance of unnecessary cordocentesis in some cases. Copyright © 2009 ISUOG. Published by John Wiley & Sons, Ltd.*

INTRODUCTION

Homozygous α-thalassemia-1 (hemoglobin (Hb) Bart's disease) is the most common cause of hydrops fetalis in South East Asia. As a result of population migrations during the past decade, this syndrome is now seen with increasing frequency in other parts of the world. The affected fetuses are almost always either stillborn or die soon after birth. Furthermore, serious obstetric complications frequently accompany affected pregnancies, including pre-eclampsia, dystocia, postpartum hemorrhage due to a large placenta, and the psychological burden of carrying a non-viable fetus to term. It is therefore important that the condition is diagnosed prenatally as early as possible, and that the parents are counseled on the option of termination of pregnancy. At present, prenatal diagnosis is usually obtained by DNA analysis or fetal blood analysis. However, these techniques are invasive and resources for analysis may be limited in areas where the disease is prevalent.

It is well established that quantification of middle cerebral artery (MCA) peak systolic velocity (PSV) is effective in predicting anemia owing to various causes[1], particularly rhesus isoimmunization[2–6], Kell alloimmunization[7], parvovirus infection[5,8,9] and placental chorioangioma[10] and that it may have a role in diagnosing Hb Bart's disease[11]. To date, there have been few studies on the role of MCA-PSV in the detection of fetal anemia secondary to Hb Bart's disease[11,12]. Moreover, the accuracy of MCA-PSV in predicting homozygous α-thalassemia-1 in fetuses in mid-pregnancy has not previously been studied. The purpose of this study was to determine the sensitivity and specificity of MCA-PSV at mid-pregnancy in the detection of Hb Bart's disease among fetuses at risk. If MCA-PSV assessment is effective in differentiating between affected and unaffected fetuses in pregnancies that are at risk, it may play an important role in identifying those that require invasive diagnostic procedures, allowing reassurance of normality in unaffected cases without unnecessary invasive intervention.

METHODS

A total of 88 pregnant women (91 fetuses) at risk of having fetuses with Hb Bart's disease opted to undergo cordocentesis to test for the disease and were recruited

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into the study, which was approved by the ethics committee. MCA-PSV was measured at 18–22 weeks of gestation in all fetuses before cordocentesis was performed at Maharaj Nakorn Chiang Mai Hospital, between May 2007 and April 2008. All ultrasound examinations, including MCA-PSV measurements, were performed by one of eight experienced perinatologists using Aloka Prosound 5000 or Aloka SSD alpha-10 (Tokyo, Japan) ultrasound machines with transabdominal 3.5-MHz curvilinear transducers. The measurement of MCA Doppler waveforms was performed using the technique described by Mari[13]. The MCA-PSV was measured from the still images of three uniform cardiac cycles using an automatic waveform analysis function integrated into the ultrasound device and the mean was calculated. Abnormal increase in MCA-PSV was defined as a value above 1.5 multiples of the median (MoM) for the specific gestational age using the nomogram for a Thai population reported by Tongsong *et al.*[14]. Definitive diagnosis was made using the gold standard technique of hemoglobin typing by high-performance liquid chromatography, following cordocentesis, in which fetuses with Hb Bart's disease typically show Hb Bart's 80–90%, Portland Hb 10–20% and a small percentage of HbH. The main outcome measure of this study was the sensitivity and specificity of MCA-PSV in the identification of Hb Bart's disease.

Table 1 Incidence of middle cerebral artery peak systolic velocity (MCA-PSV) above and below 1.5 multiples of the gestational age-specific median (MoM) in fetuses with and without hemoglobin (Hb) Bart's disease

MCA-PSV	*Fetuses with Hb Bart's disease*	*Fetuses with no Hb Bart's disease*	*Total*
> 1.5 MoM	17	0	17
≤ 1.5 MoM	3	70	73
Total	20	70	90

Sensitivity, 85.0% (95% CI, 69.1–100.0%); specificity, 100.0% (95% CI, 100.0–100.0%); positive predictive value, 100.0% (95% CI, 100.0–100.0%); negative predictive value, 95.9% (95% CI, 86.0–100.5%).

Table 2 Details of three cases with normal fetal middle cerebral artery peak systolic velocity (MCA-PSV) that were found to have hemoglobin (Hb) Bart's disease

GA (weeks)	*MCA-PSV (cm/s)*	*1.5 MoM MCA-PSV (cm/s)*	*Hb level (g/dL)*	*Degree of anemia**	*C/T ratio (%)*
18	28.2	30.9	6.9	Mild	60
19	32.6	33.0	8.7	Mild	54
20	33.4	35.1	6.5	Moderate	61

*Diagnosed using nomogram of fetal Hb level across gestational age (GA)[14]. C/T ratio, cardiothoracic ratio; MoM, multiples of the median.

RESULTS

During the study period, a total of 88 pregnant women (91 fetuses) were recruited into the study. The mean ± SD age of the population was 28.3 ± 5.7 years, ranging from 15 to 44 years, and the mean ± SD gestational age was 18.8 ± 1.1 weeks. One fetus was excluded from the study. This fetus had an increase in MCA-PSV and signs of frank hydrops fetalis. Although the fetus was provisionally diagnosed with Hb Bart's disease, fetal blood could not be obtained from cordocentesis and the fetus later died. Among the remaining 90 fetuses, the incidence of Hb Bart's disease was 22.2% (20/90).

Using MCA-PSV above 1.5 MoM as a cut-off point, the sensitivity of MCA-PSV in the detection of fetuses with Hb Bart's disease was 85.0% (17/20 cases), the specificity was 100.0%, and positive and negative predictive values were 100.0% and 95.9%, respectively (Table 1). Notably, three affected fetuses had a normal MCA-PSV, but the levels for all of them were close to the cut-off point (Table 2). Furthermore, these three fetuses also showed cardiomegaly, with an increased cardiothoracic ratio (C/T ratio) of > 50%.

DISCUSSION

In this study we determined the MCA-PSV in a group of fetuses at risk for anemia secondary to α-thalassemia-1 immediately before cordocentesis and found that a cut-off of MCA-PSV MoM > 1.5 identified 17/20 affected fetuses with a specificity of 100.0%. By using the MCA-PSV to screen for Hb Bart's disease it may have been possible for us to have avoided 70 (78%) unnecessary cordocenteses.

Although three twin pregnancies were included in this study, this does not seem to be important because the MCA-PSV in uncomplicated twin pregnancy does not differ from that in singleton pregnancy[15]. Although the MCA is a readily accessible vessel, there is still a risk of inappropriate measurement of MCA-PSV by inexperienced operators, which could negatively impact on patient outcomes[16,17]. Therefore, we have standardized the technique of MCA-PSV measurement used by the eight experienced perinatologists in order to minimize interobserver variation[13,18]. Based on this study, MCA-PSV is likely to have a role in the diagnosis of Hb Bart's disease owing to its high diagnostic accuracy. For example, for fetuses that are at risk of Hb Bart's disease but have normal MCA-PSV it seems appropriate to perform non-invasive monitoring using serial sonographic examinations to detect any hydropic change, rather than carry out invasive procedures such as cordocentesis.

Longitudinal assessment of MCA-PSV may be helpful in the detection of fetal anemia due to homozygous α-thalassemia-1 before hydropic change occurs, as seen in fetuses with rhesus isoimmunization[19,20]. However, the sensitivity in our series was somewhat lower than that in the study of fetal rhesus isoimmunization. This may be because anemia in rhesus isoimmunization was studied in late pregnancy whereas our study was performed

on fetuses earlier in gestation (mid-pregnancy) when the anemia is less apparent. We believe that all fetuses with Hb Bart's disease will show abnormal MCV-PSV in late pregnancy.

Interestingly, all of the three affected cases missed using the measurement of MCA-PSV had a relatively high MCA-PSV value close to the cut-off level of 1.5 MoM. Although the most widely accepted cut-off point for abnormal MCA-PSV is 1.5 MoM for the specific gestational age[13,21], this value was derived from many studies on populations with fetal anemia caused by isoimmunization. Therefore, it is possible that the most appropriate cut-off point of MCA-PSV for differentiating fetal anemia due to Hb Bart's disease may be below 1.5 MoM. However, the population in this study is not large enough to define the optimal cut-off point in this situation. Thus a larger study is required to demonstrate the most appropriate cut-off point of MCA-PSV for identifying Hb Bart's disease. Furthermore, all three affected fetuses with normal MCA-PSV had cardiomegaly, defined by a C/T ratio exceeding 50%. Therefore, the combination of MCA-PSV and C/T ratio may improve the sensitivity of screening for Hb Bart's disease. However, C/T ratio measurement is usually more difficult than MCA-PSV measurement and requires greater expertise.

One weakness of this study is that it may have included bias in the measurement of MCA-PSV. The color Doppler evaluation was not a blind method because the examiner could visualize the morphology of the fetuses using gray-scale sonographic imaging. Therefore, signs of early hydropic change in affected fetuses, such as cardiomegaly or pericardial effusion, could have been visible in some cases and the diagnosis of Hb Bart's in these fetuses might have been anticipated. Additionally, interobserver variation among the sonologists was not tested. On the other hand, the strengths of this study include the adequate number of cases of homozygous α-thalassemia-1 and the use of a MCA-PSV reference range constructed from our own population[14].

It is important to emphasize that normal MCA-PSV at 18 to 22 weeks reduces the probability that a fetus is affected by the disease and may obviate the need for invasive procedures to confirm normality, although follow-up sonographic examinations may be required. With this approach, invasive procedures could be performed selectively, fewer fetuses would be lost unnecessarily and medical expense could be reduced. Therefore, cordocentesis or amniocentesis should be reserved only for cases with positive sonographic markers, especially cardiomegaly. Although a small proportion of affected fetuses may be missed by this approach, it is possible that they could be detected on serial sonographic examinations because of later hydropic changes. Moreover, because the disease is almost always lethal, and termination of pregnancy can be offered at any time once a reliable diagnosis has been made, missing a very small number of cases will not greatly affect overall outcomes. Despite the high accuracy of MCA-PSV, the diagnosis should be confirmed with definitive methods such as fetal blood analysis to exclude false-positive findings, although very infrequent, and hydrops fetalis secondary to other causes.

In areas in which it is prevalent, it is important for community hospitals to select only cases at high risk for Hb Bart's disease for referral for invasive prenatal diagnosis. The measurement of MCA-PSV may represent an alternative cost-effective method in identifying cases for referral or follow-up with serial sonography in their own hospitals.

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BRIEF COMMUNICATION

Comparison of the accuracy of dichlorophenolindophenol (DCIP), modified DCIP, and hemoglobin E tests to screen for the HbE trait in pregnant women

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Beta-thalassemia/hemoglobin E (HbE) disease is an important public health problem in Southeast Asia that must be controlled. The prevalence of beta-thalassemia and *HbE* genes in our population is 3%–9% and 13%, respectively. A prenatal approach is key for effective prevention, and screening couples at risk is necessary. Effective screening methods must be developed to identity carriers. To identify beta-thalassemia carriers, the osmotic fragility test (OFT) and mean corpuscular volume (MCV) test are effective methods. For *HbE* gene carriers, the dichlorophenolindophenol (DCIP) precipitation test [1], a modified DCIP test (KKU-DCIP) [2], and the CMU-E (Chiang Mai University - HbE) test [3,4] are widely used. However, although these tests have been shown to have high accuracy, they have not been compared for their precision in the same population and they have not been evaluated when performed by inexperienced personnel. The aim of the present study was to compare the accuracy of the DCIP, KKU-DCIP, and CMU-E screening tests to identify *HbE* gene carriers.

The study was conducted prospectively between September, 2007 and June, 2008, at Maharaj Nakorn Chiang Mai Hospital, Chiang Mai, Thailand. Inclusion criteria were women presenting in the first half of their pregnancy who were not known to be a thalassemia carrier and not anemic. Exclusion criteria were women who were lost to follow-up or when data on final outcomes could not be obtained. After written consent had been obtained, 0.5–1 mL of heparinized blood was obtained from blood samples to screen for thalassemia. The laboratory techniques used were: (1) the DCIP precipitation test [2]; (2) the KKU-DCIP test, a modified DCIP test developed by Fucharoen et al. [2]; (3) the CMU-E screen test [3]; and (4) the hemoglobin A2 (HbA2) test, a simple quantitative test using microcolumn chromatography. HbA2 levels of normal, beta-thalassemia trait, HbE trait, and homozygous HbE were: <4%, 4%–9%, >10%, and >60%, respectively (HbA_2 and HbE appear in the same location of the column).

During the study period, 513 nonanemic pregnant women were recruited. Of these, 70 (13.6%) were *HbE* gene carriers. The results showed that the DCIP, KKU-DCIP, and CMU-E tests were effective in screening for HbE carriers with sensitivities of 94.3%, 94.3%, and 100.0% respectively, and specificities of 93.0%, 91.7%, and 99.1%, respectively (Table 1). The accuracy and the specificity of the CMU-E test were significantly higher than the DCIP and KKU-DCIP tests (McNemar χ^2 test; $P<0.0001$). The overall accuracy was 93.2%, 92.7%, and 99.7% for the DCIP, KKU-DCIP, and CMU-E screening tests, respectively.

The acceptable strategy for prevention and control of beta-thalassemia/HbE disease is prenatal diagnosis for couples at risk of conceiving an affected fetus [4]. As such there must be an effective and accurate method to screen for HbE carriers. Two methods used widely to screen for the thalassemia trait are mean MCV and OFT [1,4]. However, these tests do not accurately screen for HbE because about one-third of HbE gene carriers show normal screening results on both MCV and OFT [1,4]. Other methods are needed to effectively screen for *HbE* gene carriers to identify couples at risk of conceiving children with beta-thalassemia/HbE.

DCIP is used widely to screen for HbE and has a high sensitivity and specificity [5]. Other unstable hemoglobin such as HbH may result in a false-positive test. A modified DCIP test (KKU-DCIP) with a sensitivity of 98.1%–100% and a specificity of 65.4%–88.4% was proposed to reduce the false-positive rate of the conventional DCIP test [2].

Table 1
Two-by-two table showing the diagnostic indices of the DCIP, KKU-DCIP, and CMU-E screening tests to identify the hemoglobin E trait among nonanemic pregnant women (n = 513).

Test	Test result	HbE trait status		Sensitivity	Specificity	PPV	NPV	Accuracy
		Normal	HbE trait					
DCIP	Negative	412	4	94.3%	93.0%	68.0%	99.0%	93.2%
	Positive	31	66					
KKU-DCIP	Negative	406	4	94.3%	91.7%	64.1%	99.0%	92.7%
	Positive	37	66					
CMU-E	Negative	439	0	100.0%	99.1%	94.6%	100.0%	99.7%
	Positive	4	70					

Abbreviations: PPV, positive predictive value; NPV, negative predictive value.

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In our experience, interpretation of the negative and positive results from the DCIP test by evaluating the turbidity of the solution is sometimes difficult and leads to more false-positive results, whereas interpretation of the CMU-E results seems to be less problematic. Our findings using the CMU-E test were consistent with our previous preliminary report in which CMU-E showed sensitivity and specificity of 100% [3].

Based on the results of the present study, the DCIP, KKU-DCIP, and CMU-E screening tests are effective in screening for *HbE* gene carriers and are appropriate to use as primary screening tests. However, the CMU-E test will detect virtually all *HbE* gene carriers, whereas 5%–6% of carriers could be missed with the DCIP or KKU-DCIP screening tests. Furthermore, the false-positive rate with the CMU-E test is only about 1%, whereas it is approximately 7%–8% with the DCIP or KKU-DCIP test.

The strength of the present study is that the screening tests were performed using the same blood samples from one group of women, which ensured that the baseline data were the same and the accuracy of the tests could be compared. One of the limitations of the study is that the diagnostic test used was microcolumn chromatography and not DNA analysis. It has been reported that the concomitant inheritance of alpha-thalassemia often occurs and lowers the percentage of HbE.

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Sensitivity and Specificity of Mean Corpuscular Hemoglobin (MCH): For Screening Alpha-thalassemia-1 Trait and Beta-thalassemia Trait

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Objective: *To evaluate sensitivity, specificity, and positive and negative predictive value of mean corpuscular hemoglobin (MCH) for screening alpha-thalassemia-1 trait and beta-thalassemia trait*
Material and Method: *Descriptive analysis, diagnostic test, was conducted on 396 pregnant women attending the antenatal clinic between September 2007 and June 2008. Blood samples were collected from pregnant women after counseling and getting informed consent. MCH was measured in all samples by automated hematology analyzer. Determination of HbA2 level for diagnosis of beta-thalassmia trait and PCR for alpha-thalassemia-1 gene (SEA type) were performed in all cases as a gold standard. The data were collected and analyzed for sensitivity, specificity, and positive and negative predictive value of MCH for screening alpha-thalassemia-1 trait and beta-thalassemia trait.*
Results: *Based on the ROC curve, the best cut-off level of MCH in predicting the thalassemia carriers was 26.5 picrograms. Positive MCH (< 26.5 picrograms) gave the sensitivity of 95.2% and specificity of 82.3% in screening alpha-thalassemia-1 trait and beta-thalassemia trait. The positive predictive value and negative predictive value were 40.4% and 99.3% respectively.*
Conclusion: *MCH is a good tool for screening alpha-thalassemia-1 trait and beta-thalassemia trait during pregnancy because of its simplicity, low cost, (when determined as a part of complete blood count), and high sensitivity.*

Keywords: *Erythrocyte indices, Alpha-Thalassemia, Beta-Thalassemia*

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Thalassemia is the most common hematologic genetic disease in Thailand. The high prevalence of α-thal1, β-thalassemia, and HbE gene in the Thai population, 14%, 3-9%, and 13% respectively[1,2], leads to many births of children with severe thalassemia, homozygous b-thalassemia, b-thalassemia/HbE, and Hb Bart's disease. The affected persons of the first two entities have a low quality of life and have estimated average life expectancy of 10 and 30 years, respectively. Hb Bart's hydropic fetuses have never survived and their mothers often suffer from obstetric complications such as pre-eclampsia, dystocia, post-partum hemorrhage due to a large placenta, and the psychological burden for carrying a nonviable fetus to term. Each year, the authors' department faces about 20 new cases of homozygous β-thalassemia, 30-40 new cases of β-thalassemia/HbE, and 20-30 new cases of Hb Bart's disease. Therefore, these three entities of severe thalassemia need to be controlled, especially by prenatal approach[3]. Recently, the authors have had great success in the control of severe thalassemia using a simple way that cost much less than that of fetal DNA analysis[4]. However, this screening system has significant false positive test, leading to unnecessary confirmatory tests. In Thailand, the algorithm of prenatal control of severe thalassemia is diversified. For example, there are several screening tests for alpha/beta thalassemia

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such as OFT, MCV, MCH etc. Several screening tests for Hb E are available such as DCIP, KKU-DCIP, or CMU-HbE screen test. MCH is one of the screening tests for thalassemia (alpha-thalassemia1 trait and beta-thalassemia). It has not been thoroughly evaluated for its efficacy in spite of the fact that this is a simple technique and is available on an automated machine determining complete blood count in routine practice. Although, some studies showed that this might be effective in screening a thalassemia carrier, using the cut-off point at < 26-28 fl[5-7], it has never been evaluated for the best cut-off level of MCH to discriminate normal and abnormal test.

The main purpose of the present study was to evaluate the efficacy of MCH measurement in screening α-thalassemia1 and β-thalassemia carriers, using HbA2 levels measured by microcolumn chromatography and PCR for α-thalassemia1 (SEA type) as a gold standard.

Material and Method

Pregnant women attending the antenatal care clinic at Maharaj Nakorn Chiang Mai Hospital between September 2007 and June 2008 were recruited into the present study. Ethical approval was given. The inclusion criteria consisted of 1) present in the first half of pregnancies, 2) no known thalassemia carrier (history of previous child with the disease, or previous screening in the previous pregnancy), and 3) not anemic. Exclusion criteria included women with loss to follow-up or the data on final outcomes could not be obtained. All of these women were counseled and invited to join the study with informed consent. The definition used in the present study included: 1) α-thalassemia-1 carrier: Individuals who has deletion of both loci from one chromosome (--/αα). In most populations, the α-globin chain cluster or gene loci are duplicated on chromosome 16. Thus, normal genotype for diploid cells can be expressed as αα/αα. A deletion of two genes (--/αα) results clinically in α-thalassemia 1 trait, which is characterized by minimal hypochromic microcytic anemia, usually not associated with clinical abnormality and often goes unrecognized. 2) β-thalassemia carrier: Individuals who have heterozygous state of β-thalassemia gene mutation, located on chromosome 11. With beta-thalassemia carrier state, hemoglobin A2, which is composed of two α- and two δ-globin chains, is increased to more than 3.5-4%. They are usually non-anemic.

The pregnant women recruited into the present study were taken care of as a standard antenatal care. Five milliliters of blood sample was taken for MCH test as a screening test for alpha-thalassemia1 or beta-thalassemia gene carriers, HbA2 level for diagnostic test of beta-thalassemia carrier, and Polymerase chain reaction (PCR; SEA type) as a gold standard in establishing the carrier status of alpha-thalassemia1. The laboratory techniques used in the present study included: 1) Mean corpuscular hemoglobin (MCH): MCH is defined by the mean corpuscular hemoglobin measured by automated hematology analyzer (Coulter STKS analyzer; Beckman, USA) and expressed picograms. The measurement is done on a blood sample of 1.0 ml in a test tube using EDTA as an anticoagulant MCH of less than 27 pgs is considered positive or abnormal. 2) PCR for α-thal-1 (SEA type): PCR in this project is used as a definite test for detection α-thal1 gene carrier. This is a technique for amplification and analysis of DNA of α-thal1 gene, modified from Chang's method (8;9) by changing the primer specific for α-thal-1 trait. In a normal subject, PCR product will consist of only 314 base pair type, but there are PCR products of 314 and 188 base pair types in blood sample from α-thal-1 trait. 3) Hb A2 test: A simple quantitative test (using the standard A2 column kit; microcolumn chromatography) is used to identify beta-thalassemia trait. The levels of normal, β-thal trait, HbE trait, and homozygous HbE of < 4%, 4-9%, > 10%, and > 60%, respectively (HbA2 and HbE appear in the same location of the column). Used as a diagnostic test for β-trait, or HbE trait.

The main outcome was a sensitivity, specificity, and positive and negative predictive value of MCH in predicting alpha or beta thalassemia trait, using HbA2 level and PCR for α-thalassemia 1 as a gold standard.

Based on previous studies, the present study needed a sample size of at least 300 pregnant women to gain power of test 80% with confidence interval of 95%, when the sensitivity of test was more than 97% and specificity of more than 75%.

Statistical analysis included 1) demographic data or obstetric background were presented as percentage, means and standard deviation etc. 2) accuracy of the screening tests was presented as detection rate (sensitivity), and specificity with 95% confidence interval (95% CI) in detecting thalassemia carrier status.

Results

During the present study, 396 pregnant women were recruited into the present study.

Twenty-one women were excluded from the present study because of incomplete data. The remaining 375 cases were available for analysis. The majority of the subjects resided in Chiang Mai province (80.8%). Most of them were between 21-30 years old (54.5%). The occupations were mostly employee (52.2%) and housewife (27.1%). Most (54.6%) were nulliparous.

Three hundred and seventy-five blood samples were sent to measure MCH by automated hematology analyzer and all blood samples were also sent to detect beta-thalassemia trait by HbA2 test (HbA2 4.1-9%) and alpha-thalassemia-1 trait by PCR for alpha- thalassemia-1 gene (SEA type). Beta-thalassemia trait or alpha- thalassemia-1 trait was diagnosed in42 samples.

Based on ROC curve (Fig. 1), the best cut-off level of MCH in predicting the thalassemia carriers was 26.5 picrograms. Positive MCH (≤ 26.5 picrograms) gave the sensitivity of 95.2% and specificity of 83.9% in screening alpha-thalassemia-1 trait and beta-thalassemia trait. The positive predictive value was 37.9% and the negative predictive value was 99.1% (Table 1).

The positive screening test or MCH of ≤ 26.5 picograms was found in 99 samples and a negative result or MCH of more than 26.5 picograms in 276 samples. The mean (± SD) hemoglobin concentration was 12.0 ± 1.2 gm/dl. The mean (± SD) of MCH was 28.0 ± 3.3 picograms.

Of 375 pregnant women, there were 47 cases of Hb E trait (without alpha-thalassemia1 or beta carrier), which were also defined as negative final diagnosis or no thalassemia carrier. Twenty-four (55.3%) of them had a positive MCH test (≤ 26.5 picograms) and 44.7% had a negative MCH test).

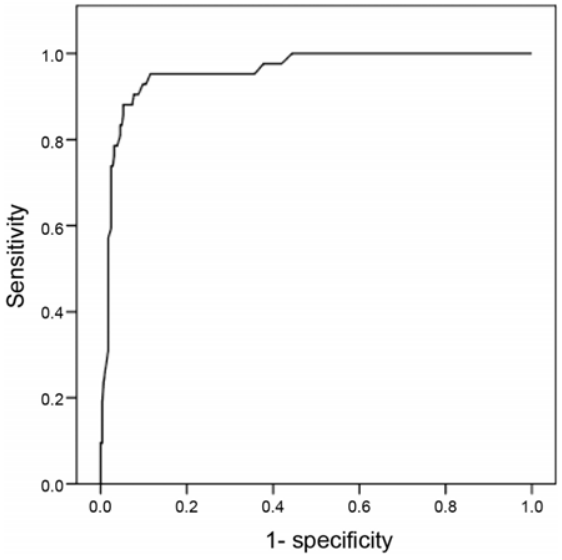

Fig. 1 ROC curve of MCH in predicting α-thalassemia-1 trait/β-thalassemia trait (area under curve 0.943)

Table 1. Two by two table shows the diagnostic indices of MCH in predicting alpha-thalalassemia1 trait or beta-thalassemia trait among non-anemic pregnant women

	α-thalassemia-1/ β-thalassemia carrier		Total
	Non-carrier	Carrier	0
MCH Negative	274	2	276
Positive	59	40	99
Total	333	42	375

Sensitivity: 95.2% (40/42) 95% [95% CI: 0.888-1.017]
Specificity: 82.3% (274/333) [95% CI: 0.782-0.864]
Positive predictive accuracy: 40.4% (40/99) [95% CI: 0.307-0.501]
Negative predictive accuracy: 99.3% (274/276) [95% CI: 0.976-1.009]

Discussion

Currently, widely used screening tests of beta-thalassemia or alpha-thalassemia-1 trait in the prevalent areas include mean corpuscular volume (MCV) and erythrocyte osmotic fragility test (EOFT). Both methods are advocated to have high sensitivity and specificity[10-12]. Maharaj Nakorn Chiang Mai Hospital used EOFT (0.45% glycerine saline solution) as a screening test of beta-thalassemia and alpha-thalassemia-1 trait[10]. However, the accuracy of MCH in screening thalassemia trait in pregnant women has not been studied. MCH could be easily screened at the first antenatal visit, using automated hematology analyzer together with complete blood count. Usually, this machine can report MCH concentration at the same time as routine complete blood count. Exclusion criteria in the present study were pregnancies that already had anemia or twins pregnancy. This was done to minimize confounding factors that may interfere with the result because many types of anemia have a direct effect on RBC volume and MCH measurement[13,14]. Moreover, in real practice, any case of anemia will completely be worked up to identify the causes including thalassemia. Therefore, there would not be any need for screening test.

Normal range of HbA2 level in the general literature was 2.5-3.5%, but it can be varied according to laboratories. In the present study, the authors used cutoff point of HbA2 level at 4%, which is the cutoff level at Maharaj Nakorn Chiang Mai Hospital Central Diagnostic Laboratory[10]. Our laboratory previously tested 128 known cases of beta-thalassemia trait that were the parents of homozygous beta-thalassemia patients and found that all of them had HbA2 > 4%, the majority of the cases ranged between 4.1-9%.

The data presented here showed that MCH at the cut-off level of ≤ 26.5 fl is a very good screening test for both beta-thalassemia trait and alpha-thalassemia-1 trait among asymptomatic pregnant women, giving a sensitivity of 95.2%, specificity of 82.3%, and negative predictive value of 99.3%. Interestingly, both false negative results in the present study were alpha-thalassemia-1 trait, indicating that MCH can screen all beta-thalassemia traits. However, positive predictive value was rather low leading to the need of the confirmatory diagnostic test in a relatively large number of women. Therefore, combination with other tests may be needed if MCH is used as a screening test.

The strength of the present study is that the MCH cut-off level used for differentiating normal from abnormal test was derived from ROC curve, unlike previous reports in which the cut-off level was based on traditional practice. Additionally, unlike previous studies that were conducted on non-pregnant participants, the present study was performed on pregnant women who may have physiologic changes of red blood cells secondary to pregnancy. Therefore, the present results may better represent the effectiveness of the test in real practice because pregnant women are the main target group of screening in prenatal control strategy for severe thalassemia.

The present study also demonstrates MCH is not effective in screening HbE trait because it has low sensitivity (55.3%). Therefore, MCH alone could not effectively detect HbE trait and an additional test such as DCIP (dichlorophenol indophenol) should be performed for such a purpose.

In conclusion, MCH is a good method for screening beta-thalassemia and alpha-thalassemia-1 trait in pregnant women. Because of its high sensitivity and specificity, along with other various aspects such as cost, availability, and technical difficulty, MCH may be one of the available techniques to be used as a primary screening test for thalassemia screening in prevalent areas. However, MCH should be determined by automated hematology analyzer that does not require more personnel or materials. Variation of the result is less than manual method that sometimes depends on personnel and the environment.

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ความไวและความจำเพาะของปริมาณเฉลี่ยของฮีโมโกลบินในเม็ดเลือดแดงในการตรวจคัดกรองพาหะแอลฟาธาลัสซีเมีย-1 และพาหะบีตาธาลัสซีเมีย

สาวิตรี พรานพนัส, สุพัตรา ศิริโชติยะกุล, เกษมศรี ศรีสุพรรณดิฐ, ธีระ ทองสง

วัตถุประสงค์: *เพื่อหาความไว ความจำเพาะ ค่าทำนายผลบวก และค่าทำนายผลลบของปริมาณเฉลี่ยของ ฮีโมโกลบินในเม็ดเลือดแดง (mean corpuscular hemoglobin: MCH) ในการคัดกรองพาหะแอลฟาธาลัสซีเมีย-1 และบีตาธาลัสซีเมีย*

วัสดุและวิธีการ: *เป็นการศึกษาเชิงพรรณนาของการทดสอบในการวินิจฉัย โดยทำการศึกษาในสตรีตั้งครรภ์ 396 รายที่มาฝากครรภ์ในโรงพยาบาลมหาราชนครเชียงใหม่ระหว่างเดือนกันยายน พ.ศ. 2550 ถึง มิถุนายน พ.ศ. 2551 ได้ทำการเก็บเลือดตัวอย่างหลังให้คำปรึกษาและยินยอมเข้าร่วมการศึกษา นำตัวอย่างเลือดไปวิเคราะห์หาระดับ MCH ด้วยเครื่องตรวจโลหิตวิทยาอัตโนมัติ และวัดระดับฮีโมโกลบิน A2 (HbA2) เพื่อวินิจฉัยภาวะบีตาธาลัสซีเมีย และตรวจพีซีอาร์ (PCR) เพื่อตรวจจีนพาหะแอลฟาธาลัสซีเมีย-1 (SEA type) นำข้อมูลมารวบรวม และวิเคราะห์หาค่าความไว ความจำเพาะ ค่าทำนายผลบวก และค่าทำนายผลลบ ในการตรวจคัดกรองดังกล่าว*

ผลการศึกษา: *เมื่อนำข้อมูลมาสร้าง ROC curve พบว่าจุดตัดของ MCH ที่ดีที่สุดในการทำนายพาหะธาลัสซีเมียดังกล่าว คือ 26.5 พิโครกรัม ซึ่งให้ความไว ความจำเพาะ ค่าทำนายผลบวก และค่าทำนายผลลบ ในการตรวจคัดกรองเป็นร้อยละ 95.2, 82.3, 40.4 และ 99.3 ตามลำดับ*

สรุป: *MCH เป็นวิธีที่ดีในการตรวจคัดกรองพาหะแอลฟาธาลัสซีเมีย-1 และบีตาธาลัสซีเมียในสตรีขณะตั้งครรภ์ เนื่องจากมีความไวสูง ทำได้ง่าย และราคาถูก*

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ORIGINAL ARTICLE

Splenic artery: peak systolic velocity of normal fetuses

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Abstract
Objective To establish a reference range of splenic artery peak systolic velocity 1(SpA-PSV) in the normal singleton pregnancies (14–40 weeks).
Methods A prospective descriptive study was conducted on uncomplicated singleton pregnancies with normal fetuses and accurate gestational age were recruited into the study. The Doppler measurements of SpA-PSV were performed by the experienced sonographers with the high-resolution machine (Aloka Prosound alpha-10, Tokyo, Japan, or Voluson E8, GE Healthcare, USA).
Results A total of 540 measurements were performed, ranging from 15 to 30 for each gestational week (GA). The best regression model between GA and SpA-PSV was observed to be linear function with an equation as follows: SpA-PSV (cm/s) = −1.433 + 1.186 (GA, weeks) ($r^2 = 0.573$). The table of nomogram for various percentile ranges was constructed. The results show a continuous increase in SpA-PSV over the period from 14 to 40 weeks.
Conclusion A nomogram for SpA-PSV for each GA during 14–40 weeks was constructed. This reference range may be a useful non-invasive tool in risk assessment for fetal anemia, especially due to homozygous alpha-thalassemia-1 or fetal isoimmunization.

Keywords Doppler velocity · Splenic artery · Ultrasound

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Introduction

With high-resolution ultrasound and pulsed/color Doppler, circulation in several fetal arteries has been effectively assessed. Ample evidence have demonstrated that fetal anemia secondary to various causes is significantly associated with several alterations in fetal hemodynamics, including middle cerebral artery as well as splenic artery circulation, which could be assessed by Doppler ultrasound [1, 2]. Bahado-Singh et al. [1, 3] showed that splenic artery peak systolic velocity (SPA-PSV) was very useful in predicting fetal anemia secondary to maternal alloimmunization [1]. Recently we have demonstrated that the mean SpA-PSV of fetuses with homozygous alpha-thalassemia-1 was significantly higher than that of unaffected ones [4], suggesting that SpA-PSV assessment may have a potential role in identifying fetuses with anemia due to homozygous alpha-thalassemia-1, though the effectiveness of SpA-PSV in differentiating affected from unaffected fetuses among pregnancies at risk needs further studies. SpA Doppler indices provide information on blood volume redistribution, e.g., an increased peak systolic velocity in fetal anemia. Like anemia due to alloimmunization, anemia in fetuses with homozygous alpha-thalassemia-1 is also associated with an increased SpA peak systolic velocity (SpA-PSV) [4]. Anemia in these fetuses can occur in early gestation, before the presence of hydropic changes visualized by ultrasound. Therefore, the assessment of SpA-PSV may enable us to make early diagnosis of homozygous alpha-thalassemia-1 in the first half of pregnancy before hydropic changes becomes overt. However, abnormal values for SpA-PSV cannot be assessed until after normal values are well established. Although the construction of gestational age-related reference curves for SpA-PSV was once reported [1], such a nomogram is included only fetuses in the second half of

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pregnancy. The nomogram of SpA-PSV for fetuses in the first half of pregnancy has never been studied, in spite of the fact that this information may be useful for early detection fetal anemia due to various causes especially homozygous alpha-thalassemia-1 in our own population. Therefore, we conducted this study to establish the reference ranges of SpA-PSV of the fetuses at gestational age of 14–40 weeks.

Materials and methods

This study was a prospective cross-sectional descriptive, performed between June 1, 2007 and March 31, 2009 at Maharaj Nakorn Chiang Mai Hospital, Department of Obstetrics and Gynecology, Faculty of Medicine, Chiang Mai University, Thailand with an approval of the research ethical committee. Low-risk pregnant women were recruited from our antenatal care unit with written informed consents. Inclusion criteria included: (1) accurate gestational age based on regular menstrual cycle with certain last menstrual period and fetal biometry in the first half of pregnancies, (2) gestational age between 14 and 40 weeks, and (3) normal pregnancy without obstetric and medical complication. Exclusion criteria included: (1) abnormal fetal growth either small-for-gestational age or macrosomia, (2) multifetal pregnancy, (3) fetal malformations, and (4) satisfactory splenic Doppler waveform could not be obtained. All ultrasound examinations were performed by the authors with real-time equipment with color and pulsed Doppler capabilities, using Aloka SSD alpha-10 (Tokyo, Japan), or Voluson E8 (GE Healthcare, USA) with transabdominal 2–4 MHz curvilinear transducers. In each examination, a routine standard sonographic examination, including fetal biometry and fetal anomaly screening, was performed. Doppler measurements were obtained at a physiologic fetal heart rate of 120–160 bpm in the absence of maternal or fetal breathing movements. The SpA-PSV was measured as follows: (1) The pregnant women were in the semi-recumbent position and the fetal abdomen was first identified in an axial view at the level of the stomach using the transducer mentioned above. (2) Color Doppler was used to locate the splenic artery as it arise from the celiac axis and courses behind the stomach into the splenic hilum (Fig. 1). (3) Pulsed Doppler evaluation was performed with a sample volume of 1–3 mm as appropriate, and an angle of insonation between 0 and 20° from the ultrasound beam. (4) The sample volume was positioned along the splenic artery in close proximity to its origin from the celiac axis. (5) Peak systolic velocity (PSV) was calculated with built-in electronic calipers. (6) Three best consecutive waveforms were selected and then automatically analyzed and averaged. (7) Repeated the above steps at least three times, the mean of them was recorded for analysis. The measurement

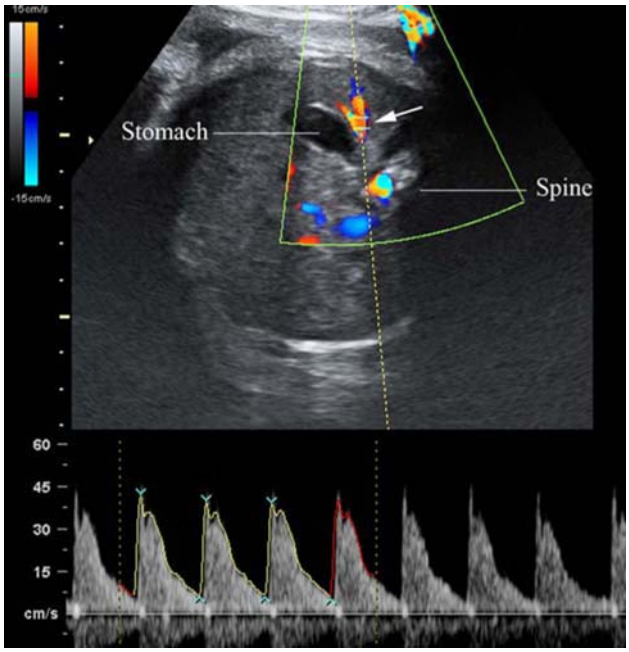

Fig. 1 Splenic artery (*arrow*) with spectral Doppler waveforms of a normal fetus at 17 weeks of gestation

usually took 5–10 min to accomplish the procedure. The collected data were analyzed for median, 5th, 10th, 90th and 95th percentiles of SpA-PSV for each gestational week (GA). The best-fit regression equation was determined using the statistical package for the social sciences (SPSS) version 17.0 (Chicago, USA).

Results

A total of 576 pregnant women were recruited into the study and 36 were excluded due to inability to obtain proper Doppler waveform (20 cases; 9 at 14–15 weeks, 5 at 16–20 weeks and 6 at 21–40 weeks), 8 fetuses with growth restriction (birth weight of less than 10th percentile), 3 cases of fetal structural or chromosome anomalies, including hydranencephaly, hydronephrosis, trisomy 21, and 5 cases of loss-to-follow-up. Of the remaining 540 measurements, the average measurement per week was 20 ± 3.4 (mean $\pm$ SD), ranging from 15 to 26 per week. The mean ($\pm$ SD) maternal age was 28 ± 5.5 years (15-42 years). Two-hundred and forty-six (45.5%) were nulliparous. Nearly all of the women were Thai ethnicity and living in the northern part of Thailand. With explore stem-and-leaf plot statistics (SPSS version 17.0), the distribution of PSV show normal in all GA. Regression analysis was used to identify the best regression model between GA and SpA-PSV which was observed to be linear equation function. The results showed the best fitted equation as follows: SpA-PSV (cm/s) $= -1.433 + 1.186$ (GA, weeks) ($r^2 = 0.573$).

Table 1 Nomogram of predicted splenic artery peak systolic velocity (SpA-PSV) at 5th, 10th, 50th, 90th, 95th percentiles for each gestational age

GA	Predicted SpA-PSV (cm/s): percentile						
	5	10	25	50	75	90	95
14	4.2	4.9	9.3	14.9	19.9	25.5	29.7
15	5.2	6.0	10.4	16.1	21.1	26.8	31.0
16	6.1	7.2	11.6	17.3	22.4	28.0	32.2
17	7.1	8.3	12.8	18.5	23.6	29.3	33.5
18	8.1	9.5	14.0	19.7	24.8	30.5	34.7
19	9.1	10.6	15.2	20.9	26.1	31.8	36.0
20	10.1	11.8	16.4	22.1	27.3	33.0	37.2
21	11.0	12.9	17.5	23.3	28.5	34.3	38.5
22	12.0	14.1	18.7	24.5	29.8	35.5	39.7
23	13.0	15.2	19.9	25.7	31.0	36.8	41.0
24	14.0	16.4	21.1	26.9	32.3	38.0	42.2
25	15.0	17.6	22.3	28.1	33.5	39.3	43.5
26	15.9	18.7	23.5	29.4	34.7	40.5	44.8
27	16.9	19.9	24.7	30.6	36.0	41.8	46.0
28	17.9	21.0	25.8	31.8	37.2	43.0	47.3
29	18.9	22.2	27.0	33.0	38.4	44.3	48.5
30	19.9	23.3	28.2	34.2	39.7	45.5	49.8
31	20.8	24.5	29.4	35.4	40.9	46.8	51.0
32	21.8	25.6	30.6	36.6	42.1	48.0	52.3
33	22.8	26.8	31.8	37.8	43.4	49.3	53.5
34	23.8	27.9	33.0	39.0	44.6	50.5	54.8
35	24.8	29.1	34.1	40.2	45.8	51.8	56.0
36	25.8	30.2	35.3	41.4	47.1	53.0	57.3
37	26.7	31.4	36.5	42.6	48.3	54.3	58.5
38	27.7	32.6	37.7	43.8	49.6	55.5	59.8
39	28.7	33.7	38.9	45.0	50.8	56.8	61.0
40	29.7	34.9	40.1	46.2	52.0	58.0	62.3

The table of nomogram for various percentile ranges was constructed (Table 1). The results show a gradual increase in SpA-PSV over the period from 14 to 40 weeks (Fig. 2).

Discussion

A few studies have been published on fetal splenic artery peak systolic velocity and they have shown that SpA-PSV may be used for the detection of fetal anemia secondary to various causes such as alloimmunization [1, 3]. Theoretically, SpA-PSV may also be useful in predicting anemia secondary to other causes such as parvovirus B19 infection, large chorioangioma or other cause of non-immune hydrops fetalis due to fetal anemia, like middle cerebral artery peak systolic velocity [5–8]. Furthermore, we have observed that fetuses with anemia due to hemoglobin Bart's disease have significantly higher SpA-PSV than that in unaffected fetuses. However, to determine whether a given SpA Doppler indices is normal or not, normal SPA Doppler indices must be established for each week of gestational age, including in early pregnancy. In addition, since these parameters may possibly be varied among different population, population-specific nomograms may be needed. Therefore, we have constructed this first nomogram of SpA-PSV for each week of gestational age, including the first half of pregnancy (14–40 weeks of gestation).

Unlike the previous report [1], which studied the SpA Doppler indices in rather late gestation, the present study has established the nomogram of SpA-PSV for each gestational age from weeks 14 to 40. We also focus on the first half of pregnancy since the main problem of fetal anemia in our population is homozygous alpha-thalassemia-1, which develops anemia from late first trimester before sonographic signs of hydropic changes become overt as seen in the second half of pregnancy. The problem of the early diagnosis is confined to the first half of pregnancy. Hopefully, if SpA-PSV facilitates early diagnosis, therapeutic termination can be earlier offered for this lethal disease, leading to avoid several serious complications related to hydrops fetalis as well as psychic trauma.

This study showed that the SpA-PSV was increased throughout the first half and second half of gestation. This finding is similar to that in the second half of pregnancy as reported by Bahado-Singh [1], who demonstrated a continuous increase of PSV of SPA over the period from 20 to 36 weeks of gestation. However, the variation of SpA indices among fetuses in each GA was rather high. Moreover, when compared to the SpA-PSV pattern in Bahado-Singh's study [1], SpA-PSV seems to be somewhat lower in every GA. This may possibly be due to the racial factors. Thus, we suggest that nomogram of SpA-PSV should be developed for their own population, especially in the area in which homozygous alpha-thalassemia-1 is prevalent.

The limitation of this study was that several cases in early gestation, 14–15 weeks, were excluded due to its difficulty in visualization of the splenic artery and proper placing the sampling gate volume. This may limit its clinical application in early second trimester. The strength of this study was based on the fact that the nomogram was constructed with adequate sample size for each the GA and reliable gestational age based on highly reliable last menstrual period and confirmation by early sonographic dating. Moreover, all examinations were done using the machines of high-resolution with experienced sonographers.

Based on our experience, to maintain the correct technique for SpA-PSV measurement, we suggest measure in a period of fetal apnea and no movement, finely adjust the transducer to have SpA visualized for its entire length, place sample volume at the SpA soon after its origin (2–3 mm), angle ultrasound beam to be close to 0°, if possible,

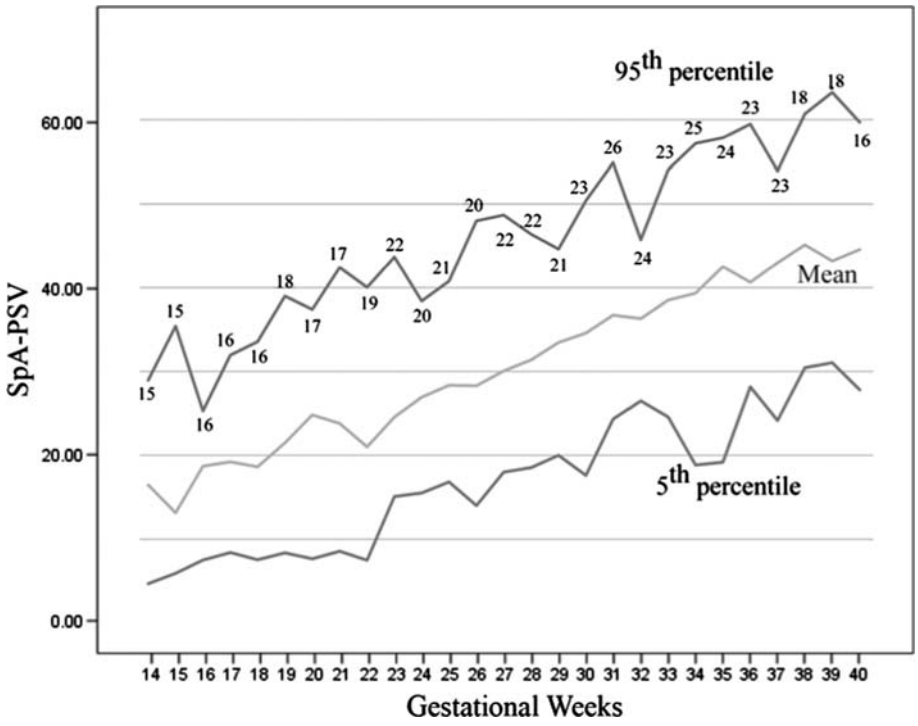

Fig. 2 Splenic artery peak systolic velocity (SpA-PSV) values for each gestational week based on raw data presented as mean, 5th and 95th percentiles (the number indicated sample size for each week)

avoiding angle corrector and finally the waveforms should be similar to each other and the highest PSV is measured.

In conclusion, a nomogram for SpA-PSV for each gestational age during 14–40 weeks is constructed. This reference ranges may be a useful non-invasive tool in risk assessment for fetal anemia in early pregnancy, such as homozygous alpha-thalassemia-1, especially where DNA testing and other diagnostic tools are not readily available. However, the effectiveness of this tool in detecting early fetal anemia needs to be tested. The future study should focus on fetuses in early pregnancy without hydropic signs to see if SpA-PSV can differentiate affected from unaffected ones before sonographic signs of hydrops fetalis become evident.

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Conflict of interest statement None.

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CLINICAL ARTICLE

A comparison of the accuracy of the corpuscular fragility and mean corpuscular volume tests for the alpha-thalassemia 1 and beta-thalassemia traits

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ABSTRACT

Objective: To compare the accuracy of the osmotic fragility test (OFT) and mean corpuscular volume (MCV) calculation when screening for the α-thalassemia 1 and/or β-thalassemia trait. *Method:* In this cross-sectional study, blood samples from 328 apparently healthy pregnant women were sent on the same day to separate laboratories for the OFT (performed using a glycerol 0.45%, phosphate-buffered, sodium chloride solution) and MCV testing (by means of a standard automated hematology analyzer). A polymerase chain reaction was also performed to positively diagnose α-thalassemia 1 carriers, and a quantitative HbA_2 test to positively diagnose β-thalassemia carriers. *Results:* Sensitivity and specificity were 95.0% and 86% for the OFT; and based on the cut-off point of 78.1 fL derived from the ROC curve, they were 93% and 93.4% for MCV calculation. The latter test was found to be slightly more accurate than the OFT in predicting the presence of the α-thalassemia 1 and/or β-thalassemia trait. *Conclusion:* Both tests have high screening sensitivity for the α-thalassemia 1 and/or β-thalassemia traits, and their simplicity and very low cost make them attractive as screening tests for large populations. Since MCV seems to provide fewer false-positive results, it may be the first choice wherever an automated hematology analyzer calculating MCV is available.

1. Introduction

Thalassemia is the most common hematologic genetic disease in Thailand. The prevalence rates of α-thalassemia 1, β-thalassemia, and *HbE* gene are high in our population (14%, 3–9%, and 13%, respectively [1,2], leading to the birth of many children with severe thalassemia, including homozygous α-thalassemia 1; Bart's hemoglobin (Hb Bart's) disease, another severe form of α-thalassemia; homozygous β-thalassemia; and β-thalassemia plus the *HbE* gene (β-thal/HbE). Although fetuses affected with Bart's hydrops syndrome (the most severe form of Hb Bart's disease) never survive, persons born with the latter two forms of thalassemia have an estimated mean life expectancy of 10 and 30 years, respectively. Along with the psychological burden of carrying a nonviable fetus to term, women carrying a fetus with Bart's hydrops syndrome often experience obstetric complications such as pre-eclampsia, dystocia, and postpartum hemorrhage due to a large placenta. Each year, our department faces about 20 to 30 cases of Hb Bart's disease, 20 cases of homozygous β-thalassemia, and 30 to 40 cases of β-thal/HbE. These 3 forms of severe thalassemia need to be controlled, especially prenatally [3]. There are several screening procedures common to α- and β-thalassemia, such as the osmotic fragility test (OFT) [4–7], mean corpuscular volume (MCV) calculation [8,9], and mean corpuscular hemoglobin calculation. Although these tests have been shown to be highly accurate under optimal laboratory conditions, they have not been compared for their precision within a given population, tested for reproducibility among groups, or evaluated when used in the real world—that is, without well-trained, highly experienced technicians. Moreover, several MCV values between 80 and 70 fL have been used as cut-off points between normal and abnormal, but the optimal value has not been rigorously determined.

The main purpose of this study was to compare 2 tests for accuracy in real-world situations, the modified OFT (where the red blood cells are placed in a glycerol, phosphate-buffered, 0.45% sodium chloride solution) and MCV calculation, both widely used to screen for α-thalassemia 1 and/or β-thalassemia. Blood samples from the same pregnant women were used for 4 tests, the OFT and MCV calculation as screening tests plus 2 gold standard diagnostic tests, polymerase chain reaction (PCR) for the α-thalassemia 1, South East Asia (SEA) trait, and HbA_2 values for the β-thalassemia trait.

2. Materials and methods

This cross-sectional analytical study was prospectively conducted between September 2007 and June 2008 after ethical approval. Pregnant women attending the Maharaj Nakorn Chiang Mai prenatal care clinic were recruited during the study. The inclusion criteria were attending the clinic during the first half of pregnancy; not being a known thalassemia carrier (i.e., having no child with the disease or having tested positive in a previous pregnancy); and not being anemic. The exclusion criteria were having other known abnormal hemoglobin types, especially the HbE

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trait, which is common in our population; being lost to follow-up; or not having outcome data available.

The α-globin chain cluster or duplicated gene loci are on chromosome 16. The normal genotype for diploid cells can be expressed as αα/αα. An individual with the deletion of both loci, and therefore 2 genes, from one of the two chromosomes 16 (- -/αα) is a carrier of the α-thalassemia 1 trait. The α-thalassemia 1 trait, which is characterized by minimal hypochromic microcytic anemia, usually is not associated with any clinical abnormalities and often goes unrecognized. An individual with a gene mutation on one of the two chromosomes 11 is a carrier of the β-thalassemia trait. Carriers of a thalassemia trait, therefore, are heterozygous for gene deletion (for those with the α-thalassemia 1 trait) or gene mutation (for those with the β-thalassemia trait). In a β-thalassemia trait carrier, adult hemoglobin A_2, or HbA_2, which is composed of 2 α- and 2 δ-globin chains, is increased to more than the normal 3.5% to 4%, and usually the carrier is not anemic.

The pregnant women recruited to the study received standard prenatal care. Blood was drawn from the participants after they gave written consent, and the samples were used for the tests.

The OFT determines the stability of red blood cells in a hypotonic solution. After 0.5 to 1 mL of blood containing EDTA as an anticoagulant was centrifuged in a 1.5-mL tube for 5 minutes at 3000 rpm. After the plasma was discarded, 10 mL of a glycerol (0.45%), phosphate-buffered, sodium chloride solution was added to 10 μL of packed red cells. Cells and solution were then mixed by inversion, and after 15 seconds the kinetics of hemolysis was recorded for 2 minutes. Hemolysis was evaluated at 20-second intervals, as the decrease in the optical density (OD) of the solution measured at 620 nm. The erythrocyte osmotic fragility value, expressed in percentage of hemolyzed cells, was calculated according to the following formula: OD at 15 seconds – OD at 120 seconds × 100/OD at 15 seconds. More than 60% of hemolyzed cells was considered normal (ie, the participant carried neither the α-thalassemia-1 nor the β-thalassemia trait). The MCV was measured using an automated hematology analyzer and expressed in femtolitres (fL). The cut-off point at which the MCV was considered abnormal was based on a receiver operating characteristic (ROC) curve derived from the data.

A diagnostic test for α-thalassemia 1 and another for β-thalassemia and β-thal/HbE were performed when both partners in the couple had positive OFT results at 2 minutes or positive MCV testing results. Polymerase chain reaction (PCR) was used as the gold standard for the definite detection of the *α-thal 1* gene of the *SEA* type. The method proposed by Chang [10,11] for DNA amplification and analysis for the *α-thal 1* gene was modified by changing the primer specific for that gene. The PCR product from blood from healthy persons consists of 314 base pairs, but the PCR product from blood from carriers of the α-thalassemia 1 consists of 314 and 188 base pairs; the latter is a result of the mutant gene on one allele. A microcolumn chromatography test measuring HbA_2 was performed for the definite detection of the β-thalassemia trait or the HbE trait. The HbA_2 levels—as a percentage of the total hemoglobin—were less than 4% for healthy individuals, between 4% and 9% for those with the β-thalassemia 1 trait, greater than 10% for those with the HbE trait, and greater than 60% for those who were homozygous for HbE (HbA_2 and HbE appearing at the same location in the column).

The main outcome was a comparison of the accuracy of the OFT and MCV calculation in detecting the α- thalassemia and/or the β-thalassemia trait, using as gold standards PCR for the α-thalassemia 1 trait and HbA_2 level for the β-thalassemia trait. Sensitivity, specificity, positive predictive value, and negative predictive value were compared.

Based on previous studies, this study required a sample size of at least 300 pregnant women to gain 80% power with a 95% confidence interval (CI) when the test sensitivity was greater than 97% and specificity greater than 75%.

Baseline characteristics of the women, all laboratory results, and fetal diagnoses, including fetal outcomes for women with normal screening results, were recorded for subsequent analysis. Unless otherwise indicated, results are presented as number and percentage or mean and standard deviation. The accuracy of the screening tests is presented as sensitivity (detection rate) and specificity in detecting carriers of a thalassemia trait.

Table 1
Results of the final diagnoses of the carrier status.

Disease type	No. of carriers (%)
α-thalassemia 1	28 (8.5)
β-thalassemia	9 (2.7)
Both types	3 (0.9)
β- thal/HbE	2 (0.6)
Total	42 (12.8)

Table 2
Two by two table showing the diagnostic indices of OFT in predicting the α-thalassemia 1 trait and/or the β-thalassemia trait among nonanemic pregnant women.[a]

OFT result	α-thalassemia 1 and/or β-thalassemia trait		Total
	Noncarrier	Carrier	
Negative	246	2	248
Positive	40	40	80
Total	286	42	328

[a] The sensitivity was 40/42 or 95.2% (95% CI, 88.8%–100%); specificity, 246/286 or 86.0% (95% CI, 82.0–90.0%); positive predictive value, 40/80 or 50.0% (95% CI, 39.0–61.0%); negative predictive value, 246/248 or 99.2% (95% CI, 97.2–100%); and accuracy, 286/328 or 87.0% (95% CI, 83.6%–90.8%).

3. Results

From September 1, 2007, to June 30, 2008, 375 women with a singleton pregnancy were recruited. Of these, 47 were later excluded because they had been diagnosed as having anemia or the HbE trait. The remaining 328 women were available for analysis. Their mean ± SD age was 27.6 + 5.4 years, their mean hemoglobin concentration was 12.4 ± 1.5 g/dL, and nearly 85% lived in Chiang Mai province.

There were 28 participants (8.5%) with positive PCR test results for the α-thalassemia-1 trait of the SEA type, and 9 participants (2.7%) had HbA_2 levels between 4.1% and 9% and were thus diagnosed as having the β-thalassemia trait (Table 1). Five of the 42 women who had an abnormal gold standard test result were positive for both the α-thalassemia-1 and β-thalassemia traits.

The pregnancy outcomes could be summarized as follows: the mean gestational age at recruitment was 38.1 ± 4.4 weeks. About two-thirds of

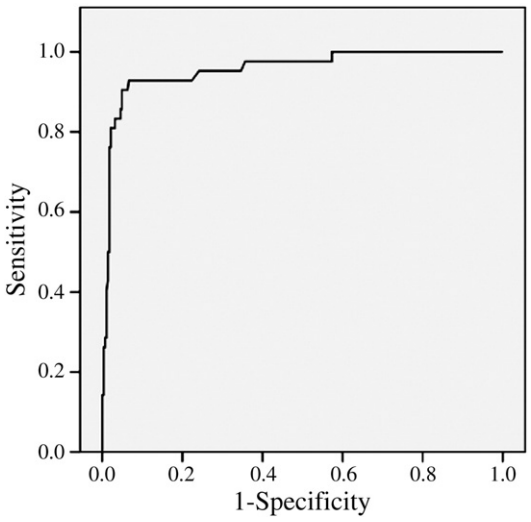

Fig. 1. ROC curve for MCV predicting α-thalassemia 1 trait and/or β-thalassemia trait (area under the curve, 0.96; 95% confidence interval, 0.93-0.99).

Table 3
Two by two table showing the diagnostic indices of MCV in predicting the α-thalassemia 1 trait and/or the β-thalassemia trait among nonanemic pregnant women.[a]

MCV result	α-thalassemia 1 and/or β-thalassemia trait		Total
	Noncarrier	Carrier	
Negative	267	3	270
Positive	19	39	58
Total	286	42	328

[a] The sensitivity was 39/42 or 93.0% (95% CI, 85.1–100%); specificity, 267/286 or 93.4% (95% CI, 89.7%–95.8%); positive predictive value, 39/58 or 67.2% (95% CI, 53.5%–78.6%; negative predictive value, 98.9% or 267/270 (95% CI: 96.2%–100%); and accuracy, 306/328 or 93.0% (95% CI, 90.6–96.0%).

the patients had a normal vaginal delivery, 14% had a cesarean delivery, and the remaining women had an assisted vaginal delivery (forceps or vacuum). The birth weight was between 2500 and 3500 g in 70% of cases.

Among the 42 carriers of severe thalassemia (whether α-thalassemia-1 and/or β-thalassemia), 40 had a positive result to the OFT screening test, for a sensitivity of 95.2% and a specificity of 86.0%. The diagnostic indices (sensitivity, specificity, positive predictive value, and negative predictive value) are presented in Table 2. Based on the ROC curve of MCV values, the best cut-off point for predicting the presence of the α-thalassemia-1 or β-thalassemia trait is 78.1 fL (Fig. 1), giving a 92.9% sensitivity and a false-positive rate of 6.6% (ie, a specificity of 93.4%). The diagnostic indices are presented in Table 3. If the traditional cut-off point of 80 fL had been used, the sensitivity would have been the same (92.9%) but the specificity would have decreased to 91.0%. Although the sensitivity of the 2 screening tests was similar ($P = 0.96$ by the χ^2 test), the specificity was significantly different ($P = 0.006$ by the χ^2 test).

4. Discussion

This study determined the accuracy of the common screening tests in detecting carriers of the α- and/or β-thalassemia traits. Along with concerns such as cost, availability, technical difficulty, its results can guide the choice of a screening test.

The incidence of severe thalassemia (β-thalassemia major and Hb Bart's disease) is very common in northern Thailand and needs to be controlled, especially prenatally [7,12,13]. However, the methods should be highly effective, simple, fast, and inexpensive. We conducted this study to evaluate the OFT as a screening test, in the hope that its high sensitivity, low cost, simplicity, and rapidity would make it the test of choice.

This study shows that the OFT has a slightly higher sensitivity but a lower specificity than the MCV test. The screening accuracy of the 2 tests was found similar, and our results imply that the carriers of severe thalassemia (whether α-thalassemia-1 or β-thalassemia) would rarely be missed with the use of either.

However, compared with the MCV test, the specificity was not as high and the false-positive rate was about 15% higher for the OFT. This means that, when using the OFT rather than the MVC test in a large population, a significant number of pregnant women would unnecessarily be further tested with more expensive tests (ie, HbA_2 concentration estimation and PCR) to confirm the screening result.

In real practice, the number of the more expensive confirmatory tests (PCR for α-thalassemia 1 carriers and the percentage of HbA_2 within total hemoglobin for β-thalassemia carriers) can be reduced if the 2 partners in the couple are screened. The definite tests for carriers are necessary only when both partners have had a positive result to a screening test. If one partner has had a negative screening result, the couple is not at risk for having a child with severe thalassemia. With this approach, the gold standard test may be needed in only 10% of the screened population.

The results of this study support the previous report on prenatal strategy to control severe thalassemia using OFT as a screening test [6,7]. We support prenatal control as follows: At the first prenatal visit, the pregnant woman and her partner will both have a blood sample taken and the woman's blood will be tested. If the result is positive, her partner's blood will be tested; but if it is negative, that is not necessary. Only when both partners have a positive result to a screening test does the diagnostic test become necessary (dealing with cases of nonpaternity, however, needs to be anticipated).

Thalassemia carriers have long been screened by the MCV test, and most institutions use 80 fL as a cut-off point. Based on our ROC curve, our study suggests the best cut-off point to be 78 fL. Choosing this cut-off point may improve screening accuracy in real practice.

Based on this study, both OFT and MCV can be used as primary screening tests for thalassemia carriers. However, we suggest that MCV be used as the primary screening test of choice wherever—along with complete blood count—it can be calculated by means of an automated hematology analyzer.

We found that as high as one-third of our pregnant population with the HbE trait had normal OFT and MCV test results (data do not shown). When screening for β-thal/HbE disease, another test should therefore be added.

The strength of this study is that both screening tests were tested with the same blood samples, thereby providing the same baseline data for both groups of carriers. This is why the accuracy of the tests could be compared. Moreover, both tests were performed in separate laboratories with no knowledge of the other's results. Unlike several previous studies conducted with nonpregnant participants, the present study compared the effectiveness of the tests in pregnant women. Their red blood cells may undergo physiologic changes; besides, pregnant women are the target group in the prenatal strategy for the control of severe thalassemia.

In summary, both OFT and MCV are highly accurate in predicting who is an α-thalassemia 1 and/or a β-thalassemia carrier, and both should be considered as highly effective screening tests, although not highly specific. Because of its high efficacy, along with its other known advantages, which include simplicity, testing rapidity, and very low cost, the OFT may be considered for the routine screening of thalassemia carriers in highly prevalent areas with limited resources. However, MCV can often be determined together with a complete blood count, and automated analyzers are frequently found at prenatal care clinics. If an automated analyzer is available, calculating MCV should be considered for the primary screening test.

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Fetal Aortic Arch Measurements at 14 to 40 Weeks' Gestation Derived by Spatiotemporal Image Correlation Volume Data Sets

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Objective. The purpose of this study was to establish reference ranges for the transverse aortic arch diameter (TAD) and distal aortic isthmus diameter (DAID) in normal singleton pregnancies (14–40 weeks) based on the 3-vessel/trachea (3VT) view of cardio–spatiotemporal image correlation (STIC) volume data sets. ***Methods.*** A prospective descriptive study was conducted on uncomplicated singleton pregnancies with healthy fetuses and an accurate gestational age (GA). Cardio-STIC examinations were performed by experienced sonographers using a high-resolution ultrasound machine, and the volume data sets were manipulated to obtain the 3VT view and measured for the TAD and DAID. ***Results.*** A total of 554 measurements were performed, ranging from 13 to 30 for each gestational week. The best regression models were as follows: TAD (in millimeters) = –1.01 + 1.69 (GA, in weeks) (r^2 = 0.93; P < .001), and DAID (in millimeters) = –0.85 + 1.54 (GA, in weeks) (r^2 = 0.92; P < .001). A table of nomograms for 5th, 50th, and 95th percentile ranges was constructed. ***Conclusions.*** Normative data for the TAD and DAID at each gestational week from 14 to 40 weeks were constructed by a new technique of measurement based on cardio-STIC. These reference ranges may be useful tools for assessment of fetal aortic arch abnormalities. ***Key words:*** aortic isthmus; fetus; 4-dimensional sonography; spatiotemporal image correlation; transverse aortic arch.

Abbreviations
CoA, coarctation of the aorta; DAID, distal aortic isthmus diameter; 4D, 4-dimensional; GA, gestational age; STIC, spatiotemporal image correlation; TAD, transverse aortic arch diameter; 3VT, 3-vessel/trachea; 2D, 2-dimensional

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In modern maternal-fetal medicine, early diagnosis of congenital heart defects, particularly in the first or early second trimester, has increasingly been given attention, and several studies have shown more cases of early detection than ever in the past. However, the total number of prenatal diagnoses of congenital heart defects is still very low worldwide. For example, the in utero detection rate of coarctation of the aorta (CoA) is very low, and it remains one of the most difficult structural cardiac abnormalities to prenatally diagnose,[1–3] although it accounts for 7% of all congenital heart defects.

To increase the detection rate of aortic arch abnormalities, measurement of aortic arch diameters has been proposed to facilitate the diagnosis. Although several nomograms of aortic arch dimensions have been published,[4–8] most included only fetuses in the second half of pregnancy, used machines in the era of low-resolution sonography,[5] or had problems with small sample sizes.[4] Moreover, because fetal size varies in different regions of

the world, it would be more appropriate for each to have its own normal reference ranges. Nomograms for earlier gestation than in the past are also needed because of an increased need for early diagnosis, especially in cases of thickened nuchal translucency.[9] Therefore, normal ranges for the transverse aortic arch diameter (TAD) and distal aortic isthmus diameter (DAID) are desirable.

With modern high-resolution ultrasound equipment featuring spatiotemporal image correlation (STIC) volume data sets and compound imaging capabilities, which allow for off-axis beam steering as an asset, we can measure the aortic dimensions more accurately than in the past. Therefore, we aimed to construct normal reference ranges for the TAD and DAID at 14 to 40 weeks' gestation with a high-resolution machine using an offline STIC technique, which allowed us to rotate the cardiac volume data sets to control the axis and establish the exact dimensions of the transverse aortic arch and distal aortic isthmus. It is hoped that our nomograms may be helpful for prenatal diagnosis of congenital heart defects, including abnormal growth of the aortic arch.

Materials and Methods

This prospective cross-sectional descriptive study was performed between September 1, 2007, and May 31, 2009, at the Department of Obstetrics and Gynecology (Maharaj Nakorn Chiang Mai Hospital), Faculty of Medicine, Chiang Mai University. Pregnant women attending our antenatal care clinic were recruited into the study with written informed consent. Inclusion criteria were (1) an accurate gestational age (GA) based on regular menstruation with the exact date of the last menstrual period and fetal biometric measurements in the first half of pregnancy; (2) a GA between 14 and 40 weeks' gestation; and (3) low-risk pregnancy without known obstetric or medical complications. Exclusion criteria were (1) abnormal fetal growth, including small size for GA (<10th percentile for each gestational week) and macrosomia (>90th percentile); (2) fetal malformations; (3) multifetal pregnancy; and (4) inability to obtain a satisfactory 4-dimensional (4D) STIC volume.

All sonographic examinations were performed by experienced perinatologists using real-time equipment with 4D STIC (Voluson E8; GE Healthcare, Milwaukee, WI) and transabdominal 2- to 4-MHz curvilinear transducers. In each examination, sonography, including fetal anomaly screening and fetal biometric measurements, was first performed. To identify the transverse aortic arch and distal aortic isthmus, the 4D STIC examination was performed as described below.

Volume Acquisition

Volume data sets were obtained from each participant by a transverse sweep after the best 4-chamber view was obtained, preferably acquired with the cardiac apex oriented anteriorly and a 10° to 40° angle between the interventricular septum and ultrasound beam. The volumes were acquired in the absence of fetal movement, with the participant momentarily suspending breathing during the volume acquisition, which lasted 7.5 to 12.5 seconds. After the acquisition, the participant could go home, and the virtual cardiac cycle volume was recorded in the hard drive for subsequent offline examination.

Volume Processing

The volume data sets underwent image processing, including adjustment of brightness and contrast to optimize tissue contrast resolution and color filtering as appropriate for analysis.

Offline Volume Data Set Analysis

Multiplanar slice analysis was performed after the participant left the examination room, with the objective of performing basic and extended cardiac examinations, as recommended by the American Institute of Ultrasound in Medicine,[10] to rule out any congenital cardiac defects. Dynamic images of the fetal heart were simultaneously visualized in the 3 orthogonal planes (axial, coronal, and sagittal). Images of the 4-chamber view, outflow tracts, aortic arch, and ductal arch were navigated and displayed by moving the reference dot upward and rotating the fetal heart around the 3 orthogonal axes (x, y, and z)

Transverse Aortic Arch and Distal Aortic Isthmus Imaging

The planes of the TAD and DAID were shown by rotating the volume and scrolling the reference dot to display both the transverse aortic arch and

distal aortic isthmus in plane A, keeping the transverse aortic arch exactly vertical, and the descending aorta in plane C in the same alignment as in plane A, resulting in a sagittal view of the aortic arch in plane B (Figure 1).

Transverse Aortic Arch and Distal Aortic Isthmus Diameter Measurement

By freezing the volume slice at the time of peak systole, giving the greatest aortic arch dimension, and measuring the greatest dimensions of the TAD and DAID in plane A, the points of ductal joining and neck artery branching could be easily seen in planes A and B, respectively. With the 3-vessel/trachea (3VT) view in plane A, the TAD was measured between the common carotid and the left subclavian arteries, and the DAID was measured distal to the origin of the left subclavian artery, both during ventricular systole. Three best measurements were selected, averaged, and recorded for subsequent analysis. The collected data were analyzed for the median and the 5th, 50th, and 95th percentiles of the TAD and DAID at each gestational week. A best fit regression equation was determined with SPSS version 17.0 software (SPSS Inc, Chicago, IL). The first 50 measurements were performed by all of the authors for evaluation of interobserver variations.

Results

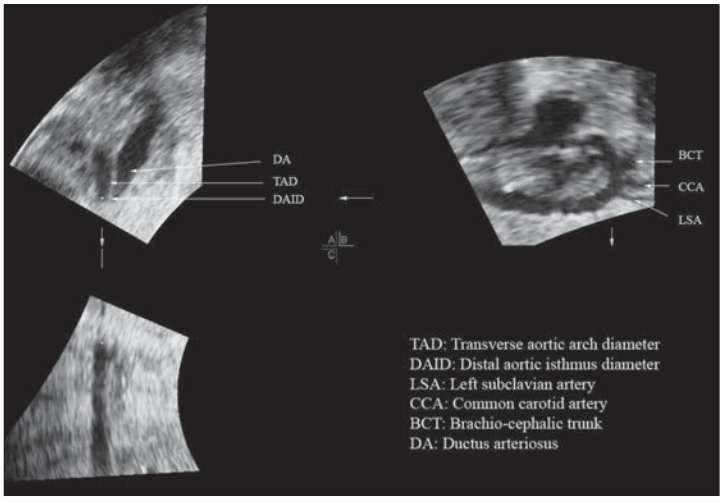
A total of 598 pregnant women were recruited into the study, and 44 were excluded because of an inability to acquire good-quality volume data sets (most were at 14–15 weeks' gestation), fetuses with growth restriction (birth weight <10th percentile), and fetal structural or chromosomal anomalies, including congenital heart defects (5), omphalocele (2), and a diaphragmatic hernia (1). Of the remaining 554 measurements, the average number of measurements per week was 20.5, ranging from 13 to 30. The mean maternal age ± SD was 27.1 ± 5.8 years (range, 15–43 years). Two hundred fifty-nine (46.75%) were nulliparous.

For both the TAD and DAID, the internal diameter was found to increase in a linear fashion throughout the second and third trimesters (Figure 2). The distributions of the TAD and DAID were normal at all gestational weeks. Regression analysis was used to identify the best regression models for their relationships. The results showed the following best fitted equations: TAD (in millimeters) = –1.01 + 1.69 (GA, in weeks) (r^2 = 0.93; P < .001), and DAID (in millimeters) = –0.85 + 1.54 (GA, in weeks) (r^2 = 0.92; P< .001). A table of nomograms for 5th, 50th, and 95th percentile ranges for the TAD and DAID was constructed (Table 1). The results show a gradual increase in both the TAD and DAID from 14 to 40 weeks (Figure 2). The interobserver mean differences for measurement of the TAD and DAID were 2.6% and 2.7%, respectively, and the intraobserver mean differences were 2.1% and 2.3%. Of interest, during the study period, 3 cases of fetal CoA were encountered at 19, 22, and 33 weeks' gestation. All of them had TAD and DAID measurements below the 5th percentile.

Discussion

Although several studies on the TAD and DAID have been published, this study is unique and different from other previous studies in the aspect of the planes for measurement, which were based on cardio-STIC. Furthermore, this study included pregnancies earlier in gestation, a larger sample size, and examinations with a high-resolution ultrasound machine. To our best knowledge, normative data for the aortic arch derived from healthy pregnancies based on eval-

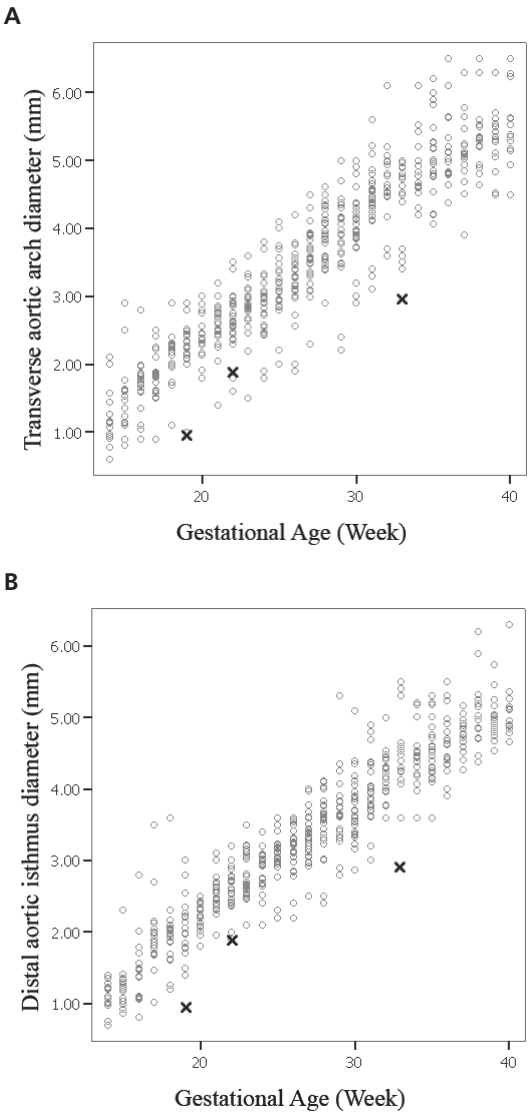
Figure 1. Example of the 3 orthogonal plane views for evaluation of the aortic arch with cardio-STIC.



uation of the 3VT view in cardio-STIC volume data sets have not been published previously, and they may be helpful for prenatal diagnosis of congenital heart defects, including abnormal growth of the aortic arch.

Although several views and planes in the cardio-STIC volume data set mode could be visualized for measurement of the TAD and DAID, we preferred measurement in the 3VT view in plane A for several reasons. In standard cardio-STIC, the 3VT view has been recommended as an essential view for evaluating cardio-STIC data sets.[11]

Figure 2. Scattergrams of the TAD (**A**) and DAID (**B**) for each gestational week. The cross marks represent values from fetuses with CoA.



The 3VT view is a cross-sectional view of the upper mediastinum, usually obtained by scrolling the reference dot upward from the 4-chamber view. In real practice, the acquired volume data sets are usually based on the 4-chamber view on 2-dimensional (2D) sonography. With this acquisition, the 3VT view is associated with better resolution than the sagittal aortic arch view, whereas the sagittal aortic arch view gives better resolution with acquisition in a sagittal scan of the fetal chest. Because in common practice most sonographers prefer to perform cardio-STIC with acquisition in the 4-chamber view, we chose to establish normative data for TAD and DAID reference rages in the 3VT view to be more clinically applicable.

The accuracy of the measurements was theoretically better than that of those derived from 2D sonography. This is due to the fact that with cardio-STIC, we could rotate the volume data set and move the reference dot in 3 orthogonal planes to control the display of the aortic arch to be exactly vertical in plane A, the descending aorta exactly along the coronal view in plane C, and the midsagittal view in plane B. Visualization of the 3 orthogonal planes at the same time can ensure the correct position for measurement.

With the multiplanar mode, during measurement in plane A, we could exactly specify the point for measurement by showing the branching point of the common carotid and left subclavian arteries in plane B and the juncture of the ductus arteriosus and descending aorta in plane C. This allowed us to identify the position of the TAD (the portion located between the left common carotid and left subclavian arteries) in plane B and measure it in plane A. The DAID (the portion located between the left subclavian artery and ductal entry) could be exactly identified by showing the left subclavian artery in plane B and the ductal entry in plane A, which is different from measurement by 2D sonography, in which these relationships cannot be shown on the same image at the same time. Some authors have preferred to measure in the sagittal view of the aortic arch, in which the neck vessel could be easily seen,[4,5] but in this view, the juncture point of the ductus arteriosus and descending aorta could not be seen. Notably, the location of the TAD in the 3VT view (plane A) will be represent-

ed by that reference dot at the same level in plane B, where the dot is located between the left common carotid and left subclavian arteries, but the measurement is performed in plane A because the resolution in plane A is better than that in plane B.

Of interest, both the TAD and DAID in this study were somewhat smaller than those in western reports.[4–6] This may possibly have been associated with racial factors and was consistent with the findings reported by Noomcharoen and Uerpairojkit,[12] implying a need for population-specific nomograms.

In the future, cardio-STIC has great potential to be useful in confirmation of various cardiac defects, including evaluation of the aortic arch. We believe that the normative reference ranges derived from cardio-STIC may be more appropriate for evaluation and may facilitate diagnoses earlier in gestation.

In addition to the large sample size and examination with a high-resolution ultrasound machine, another strength of this study was that it was highly reliable because each measurement was performed by an offline examination without a time limit, allowing us to obtain the best image. Moreover, the interobserver variations in the measurements were acceptable.

Limitations of this study were that the resolution of the virtual cardiac cycle may have been somewhat decreased in several cases secondary to maternal or fetal movement, and it was impossible to obtain good acquisitions in some cases with very active fetal movement for a long period or fetuses in positions where the heart was poorly accessible. Additionally, even with a high-resolution ultrasound machine, the small structures of the aortic arch could not be clearly visualized in the early second trimester.

In conclusion, we constructed nomograms for the TAD and DAID at 14 to 40 weeks' GA. These reference ranges may be useful tools for assessment of the fetal heart even in early pregnancy, especially when CoA is suspected. However, the effectiveness of these tools in detecting early fetal CoA needs to be tested. This study also used a new technique for measurement of the TAD and DAID in cardio-STIC volume data sets.

Table 1. Calculated TAD and DAID at the 5th, 50th, and 95th Percentiles for Each GA

		TAD, mm			DAID, mm		
GA, wk	n	5th	50th	95th	5th	50th	95th
14	15	0.818	1.356	1.980	0.783	1.306	1.815
15	15	0.963	1.525	2.172	0.913	1.460	1.992
16	17	1.108	1.694	2.364	1.043	1.614	2.169
17	18	1.253	1.863	2.556	1.173	1.768	2.346
18	19	1.398	2.032	2.748	1.303	1.922	2.523
19	18	1.543	2.201	2.940	1.433	2.076	2.700
20	16	1.688	2.370	3.132	1.563	2.230	2.877
21	22	1.833	2.539	3.324	1.693	2.384	3.054
22	26	1.978	2.708	3.516	1.823	2.538	3.231
23	27	2.123	2.877	3.708	1.953	2.692	3.408
24	23	2.268	3.046	3.900	2.083	2.846	3.585
25	23	2.413	3.215	4.092	2.213	3.000	3.762
26	25	2.558	3.384	4.284	2.343	3.154	3.939
27	30	2.703	3.553	4.476	2.473	3.308	4.116
28	26	2.848	3.722	4.668	2.603	3.462	4.293
29	23	2.993	3.891	4.860	2.733	3.616	4.470
30	29	3.138	4.060	5.052	2.863	3.770	4.647
31	28	3.283	4.229	5.244	2.993	3.924	4.824
32	20	3.428	4.398	5.436	3.123	4.078	5.001
33	16	3.573	4.567	5.628	3.253	4.232	5.178
34	18	3.718	4.736	5.820	3.383	4.386	5.355
35	21	3.863	4.905	6.012	3.513	4.540	5.532
36	19	4.008	5.074	6.204	3.643	4.694	5.709
37	17	4.153	5.243	6.396	3.773	4.848	5.886
38	16	4.298	5.412	6.588	3.903	5.002	6.063
39	14	4.443	5.581	6.780	4.033	5.156	6.240
40	13	4.588	5.750	6.972	4.163	5.310	6.417

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Effectiveness of Prenatal Control of Severe Thalassemia

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Effectiveness of Prenatal Control of Severe Thalassemia

Abstract

Objective: To determine the effectiveness of prenatal strategy in reducing new cases of severe thalassemia

Design: A prospective analytic study

Subjects: A symptomatic pregnant women attending antenatal clinic at six tertiary centers

Methods: The strategy included: 1) carrier identification using MCV as a screening test for thalassemia (alpha-thal-1 and beta-thal) and CMU-E screen as a screening test for HbE carrier, and HbA2 level measurement as a confirmatory test for beta-thal and HbE carrier and IC strip / PCR as a confirmatory test for alpha-thal-1 trait; assemia carrier and 2) the couples at risk were counseled and offered prenatal diagnosis either DNA-based (CVS, amniocentesis) or fetal blood analysis (cordocentesis), or serial ultrasound, as appropriate; and 3) counseling for termination of pregnancy in case of affected fetus. All neonates were evaluated for thalassemia status.

Results: Of 8247 pregnant women were recruited, 132 couples were identified to be at risk of fetal severe thalassemia and 128 underwent prenatal diagnoses. Of them, 32 affected fetuses were identified and pregnancies were terminated. A total of 5125 neonates among couples of no risk were also evaluated and none of them was affected. The strategy had sensitivity and specificity of 100% and 25%, respectively. The strategy could detect all of affected fetuses among 5243 fetuses with known final diagnosis.

Conclusion: The strategy could prenatally identify affected fetuses with a detection rate and negative predictive value of 100%. However, false positive was about 75%, consistent with theoretical risk of having affected fetuses 25%. Overall, we conclude that the strategy are very effective and are able to prenatally detect all affected fetuses with an acceptable false positive rate.

Effectiveness of Prenatal Control of Severe Thalassemia

Introduction

Thalassemia is the most common hematologic genetic disease in Thailand. The prevalence of α-thalassemia-1, β-thalassemia, and HbE gene in our population is as high as 14%, 3-9%, and 13% respectively(1;2), leading to many births of children with severe thalassemia, including homozygous β-thalassemia, β-thalassemia/HbE, and Hb Bart's disease. The affected persons of the first two entities have low quality of life and have estimated average life expectancy of 10 and 30 years, respectively while Hb Bart's hydropic fetuses have never survived and their mothers often suffer from obstetric complications such as pre-eclampsia, dystocia, postpartum hemorrhage due to a large placenta, and the psychological burden for carrying a nonviable fetus to term. Each year, our department faces about 20, 30-40, and 20-30 new cases of homozygous β-thalassemia, β-thalassemia/HbE and Hb Bart's disease, respectively. Therefore, these three entities of severe thalassemia need to be controlled, especially by prenatal approach(3). Currently, a number of screening tests have been developed to identify the individuals with higher risk of thalassemia carrier and they will be confirmed for carrier status with various tests, including DNA-based or non-DNA-based diagnosis. Likewise, fetal diagnoses are also either DNA or non-DNA based techniques. Several studies on fetal DNA analysis have been reported(3-7). The main advantage of this approach is the possibility of an early prenatal diagnosis from 10-14 weeks of gestation onwards by analysis of chorionic villi. However, a drawback is high cost, requirement of much technical effort and impossibility to be widely available, especially in rural areas. To date, obviously fetal DNA analysis is attractive but not effective in prevention of severe thalassemia in larger areas, especially in developing countries. Therefore, the more feasible methods should be sought for. Recently we have a great success in control of severe thalassemia with simple way with much lower cost than that of DNA-based analysis(8). This screening system may have significant false positive test, leading to unneccessary cordocentesis. We have launched the strategy for prenatal control of severe thalassemia in our hospital for several years. However, though we have success with preliminary project, several problems need to be solved. For example, the strategy allowed us making diagnosis of severe thalassemia rather late in gestation, 18-22 weeks. Furthermore, we have not known whether the strategy is effective in term of detection rate and false negative rate (the number of affected cases missed from the program).

In Thailand the algorithm of prenatal control of severe thalassemia is diversified. For example, there are several screening tests for alpha / beta thalassemia including OFT, MCV, MCH etc. Several screening tests for Hb E are available such as DCIP, KKU-DCIP(9), or CMU-E screen test(10). Though our strategy in prevention and control has been highly successful(11), we have changed some parts of the strategy, either screening

tests or prenatal invasive techniques, according to new accumulating evidence. Based on our recent project regarding comparison of various tests, we found that MCV is most cost-effective for large scale screening(12) and CMU-E screen is the best screening method for Hb E carrier screening(13). In addition, we have also found that simple and inexpensive immuno-chromatographic strip test for diagnosis of alpha-thal1 carrier has high sensitivity (100%) and high specificity (89%)(14). This test is highly effective in identifying cases with abnormal screening thalassemia screening for further confirmation with PCR technique. With this approach, more than two-third of PCR tests can be avoided.

Based on long-term and extensive studies, we have developed the strategy of prenatal control of severe thalassemia including specific screening tests (MCV and CMU-E screen with various options of prenatal diagnosis; CVS, amniocentesis, cordocentesis and ultrasound examinations. However, the effectiveness of the strategy has never been completely tested. Several studies, including our large studies, have shown high prenatal detection rates of severe thalassemia but none has studied on couples with negative screening test and we have never known how many new cases occur during the period of prenatal control strategy. Therefore, we conducted this studies mainly to evaluate the effectiveness of our strategy of prenatal control of severe thalassemia (Hb Bart's hydrop fetalis, homozygous β-thalassemia, and β-thalassemia / HbE), by testing in large-scaled population to determine the detection rate as well as new cases of severe thalassemia occurring in the project (false negative). Therefore, in this new project, all fetuses of screened mothers, either negative or positive screening tests, would be evaluated for thalssemia statuses.

Materials and Methods

This prospective study was conducted in six tertiary centers in Thailand with institute ethical approval and financial supported by Thailand Research Fund (TRF) and the Commission on Higher Education (CHE) of Thailand. The strategy consisted of genetic counseling, identification of pregnancy at risk, prenatal diagnosis (cordocentesis and fetal blood analysis), and termination of affected pregnancy, as shown in the box and figure 1. Carrier identification will be performed by prospective screening (screening program for women at no known risk). Options for definite prenatal diagnosis for fetuses at risk includes chorionic vilous sampling at 10-14 weeks of gestation or amniocentesis at 16-18 weeks of gestation or cordocentesis followed by fetal blood analysis with HPLC (High Performance Liquid Chromatography) at 18-22 weeks of gestation or serial ultrasound; depending on gestational age of known results, availability of prenatal service, type of thalassemia risk, and couples' preference. Termination of pregnancy is done with conventional methods of each center and the postabortal blood analysis is done to confirm the prenatal diagnosis.

Study population was pregnant women attending antenatal care clinic at each center described above between August, 2008 and December, 2010. **Inclusion Criteria:** 1) attending antenatal care clinic in the first half of pregnancies, 2) not anemic, defined by hemoglobin > 10 gm/dl. **Exclusion Criteria:** 1) known cases of thalassemia carrier, or loss to follow-up or the data on final pregnancy outcomes could not be obtained

Some definitions used in this study included:- **α-thalassemia-1 carrier:** individuals who had deletion of both loci from one chromosome (- -/αα) or Southeast Asian Type (SEA); **β-thalassemia carrier;** individuals who had heterozygous state of β-thalassemia gene mutation, located on chromosome 11, either β^0 or β^+. With β-thalassemia carrier state, hemoglobin A2 was increased to more than 4%, usually non-anemic; **HbE trait:** Individuals with heterozygous state of HbE gene mutation (gene coded for codon 26 of β-globin chain, codon GAG mutated to AAG), located on chromosome 11. With HbE trait or carrier state, hemoglobin E accounts for 25-30% of all hemoglobin; **Severe thalassemia diseases (syndromes):** homozygous α-thalassemia-1 (Hb Bart's disease), homozygous β -thalassemia (β-thalassemia major) and β -thalassemia/HbE diseases; **Mean corpuscular volume (MCV):** the mean corpuscular volume measured by automated hematology analyzer and expressed in femtolitre unit, considered abnormal when the measurement of less than 78 fl(15); **CMU-E-screen test:** A technique used to screen individual who is HbE carrier, using microcolumn DEAE Sephadex A50 chromatography(10;16); **Polymerase chain reaction (PCR) for α-thal-1 (SEA type)**: a technique for amplification and analysis of DNA for α-thal-1 gene, modified from Chang's method(17;18) by changing the primer specific for α-thal-1 trait; **Alpha-Thal IC Strip Test:** A strip test using monoclonal antibody sandwich technique to identify minute amount of Hb Bart's in maternal blood(19), used as a secondary screening test in couples with positive MCV, for selecting cases to do PCR; **Hb A2 test:** A simple quantitative test (using standard A_2 column kit; microcolumn chromatography) is used to identify beta-thalassemia trait. The levels of normal, β-thal trait, HbE trait, and homozygous HbE are < 4%, 4-9%, >10%, and >80%, respectively (HbA_2 and HbE appear in the same location of the column). Used as a diagnostic test for β-trait, or HbE trait in the couple of positive MCV or positive HbE screen; **Isoelectric focusing (IEF):** an electrophoretic technique with excellent resolution used to identify and quantify hemoglobin in neonates.

Steps in the strategy: 1) Women meeting the inclusion criteria were invited to the study and taken care as a standard antenatal care. When the written consent was obtained, blood sample from the women as well as the husbands would be taken for screening test of thalassemia. 2) All recruited women were counseled for screening at first visit and then two ml of blood were obtained and tested for screening test and diagnostic test as needed. The prospective algorithm is shown in figure 1. The couple at risk for having a baby with severe thalassemia was a couple who both were α-thal-1 trait, both were β-trait, or one was β-trait while the other was HbE trait. They were at risk for having a child with Hb Bart's disease, homozygous β-thalassemia or β-thalassemia/HbE disease, respectively. In practice, we collected blood from both of the couple at first visit, if both come together. Most screened women had negative MCV and E-screen test and needed no husband testing. If the woman was positive, the husband would be tested. If one of them had negative test, they were not a couple at risk. Only when both had positive respective test, diagnostic test would be done. Techniques for MCV measurement(15) and CMU-E-screen test(20) were described elsewhere. 3) Pregnant women with negative both tests would be managed as routine antenatal care. The pregnant women with positive for either test would be asked for taking blood sample of her husband for the same screening (if the husband did not come at the first visit). If the results indicated that the couples were at

risk for having fetuses with Hb Bart's disease, beta-thalassemia major or beta-thalassemia / HbE disease, the confirmatory test for carrier status were performed , Hb A 2 levels measurement for beta-thal1 trait or HbE trait **AND** alpha-thalassemia immunochromatographic strip test (IC Strip) for alpha-thal1 trait. If IC strip was negative, alpha-thal1 trait could be excluded and if IC strip was positive, alpha-thal1 trait would be confirmed by polymerase chain reaction or PCR technique (α-thal-1; SEA type); 4) Pregnant women with no risk were managed as routine antenatal care. If they were at risk for beta-thalassemia major or beta-thalassemia / HbE, chorionic villous sampling or amniocentesis or cordocentesis as appropriate for fetal diagnosis were offered. If fetuses were at risk for Hb Bart's disease, the options of serial ultrasound every two weeks until term or invasive diagnosis would be offered. In cases of serial ultrasound, when sonographic markers of fetal anemia appear, invasive diagnosis, e.g. cordocentesis, for definite fetal diagnosis would be offered. 5) The pregnancies with unaffected fetuses were managed as routine antenatal care. The pregnancies with affected fetuses were offered the choices of continuing pregnancy in high risk clinic or termination of pregnancy. 6) All newborns were taken blood (cord blood after birth) for hemoglobin typing analysis with IEF.

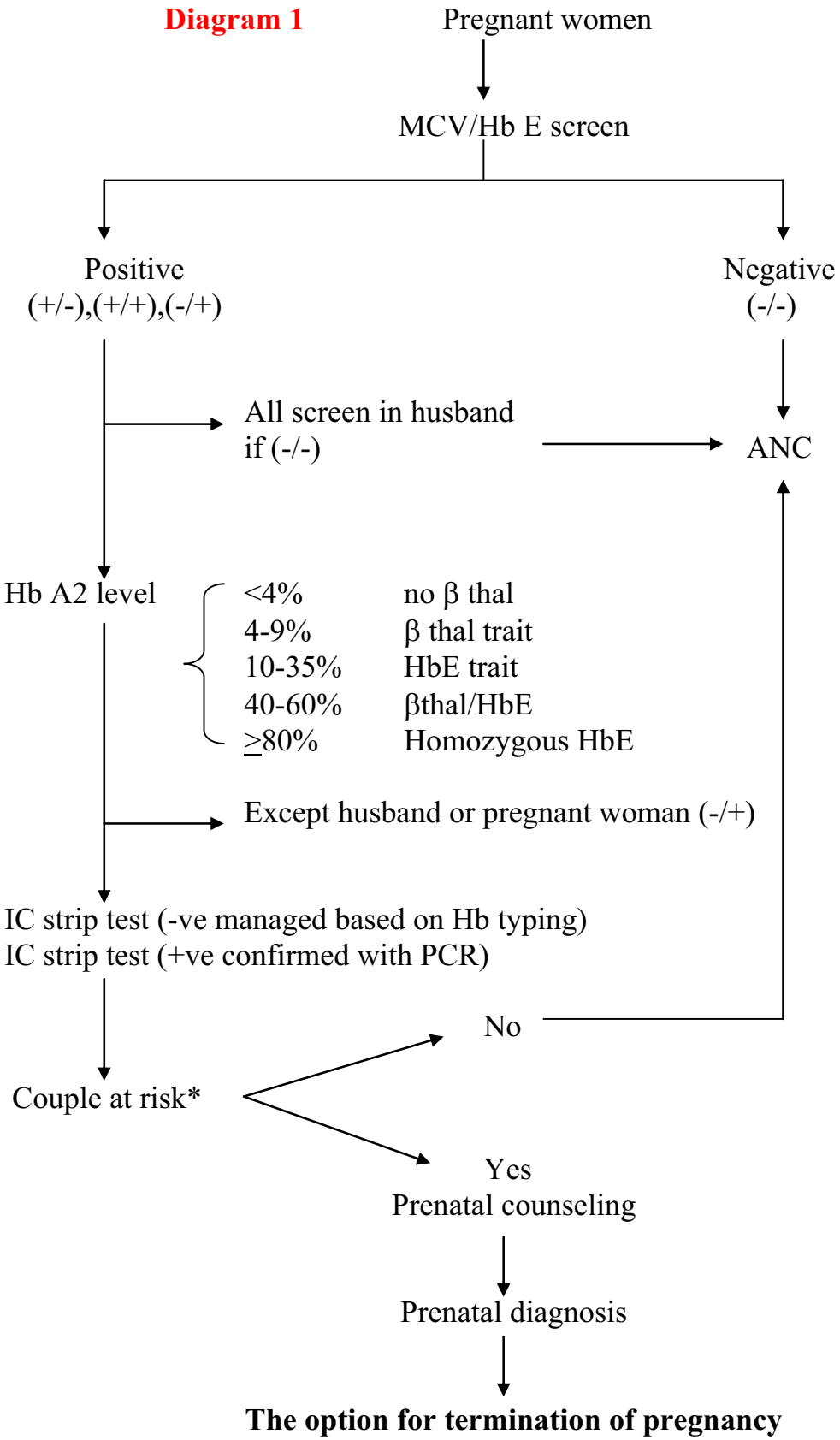
The Main Strategy

1) Genetic counseling
2) Identificaion of pregnancies at risk with prospective screening
   Screening test
   1. MCV screening for thalassemia trait (alpha-thal-1, or beta-thal)
   2. CMU-E screen for HbE trait
   Diagnostic test (if both of the couple were positive screening test)
   1. positive for thalassemia trait screening:
      HbA_2 (Microcolumn)
      Diagnosis for α-thal-1 (IC strip/ PCR)
   2. positive for CMU-E screen only: (risk for HbE trait but not no risk of)
      Diagnosis for beta- and HbE trait by HbA_2 level (Microcolumn)
3) Prenatal diagnosis for fetuses at risk
   Prenatal counseling & Prenatal diagnosis
   1 Risk for beta thalassemia major, beta-thalassemia / HbE disease
   - Invasive diagnosis (CVS or Amniocentesis or Cordocentesis (for fetal DNA or fetal blood analysis as appropriate)

   2 Risk for Hb Bart's disease
   - Invasive diagnosis OR
   - Ultrasound at first visit (after 12 weeks)
     - No ultrasound marker: repeat ultrasound every 2 weeks
     - +ve for ultrasound marker: invasive fetal diagnosis

4) Counseling and termination of affected pregnancy
5) All newborns recruited into the project will undergo hematologic studies with IEF

Diagram 1


Pregnant women
MCV/Hb E screen
Positive
(+/-),(+/+),(-/+)
Negative
(-/-)
All screen in husband
if (-/-)
ANC
Hb A2 level
<4% no β thal
4-9% β thal trait
10-35% HbE trait
40-60% βthal/HbE
≥80% Homozygous HbE
Except husband or pregnant woman (-/+)
IC strip test (-ve managed based on Hb typing)
IC strip test (+ve confirmed with PCR)
No
Couple at risk*
Yes
Prenatal counseling
Prenatal diagnosis
The option for termination of pregnancy

*Couple at risk

Homozygous β thalassemia:	β thal trait and β thal trait
	β thal trait and β thal / HbE
β thalassemia / HbE :	β thal trait and HbE trait
	β thal trait and homozygous HbE
	β thal trait and β thal/HbE disease
Hb Bart's diease	α thal 1 trait and α thal 1 trait

Statistical analysis: Demographic data or obstetric background would be presented as percentage, means and standard deviation etc. Effectiveness of the prenatal control strategy for severe thalassemia would be presented as detection rate (sensitivity) and specificity in detecting fetuses with severe thalassemia syndrome

Results

A total of 8247 pregnant women were prospectively screened in six tertiary centers as shown in Table 1. Of them, 2668 cases had a positive screening test and finally 132 couples were identified to be a couple at risk of severe thalassemia. Of these fetuses at risk, 128 underwent prenatal diagnoses by chorionic villous sampling (37) cordocetesis (45 cases), ultrasound (for fetuses at risk of Hb Bart's disease; 40 cases), amniocentesis, 6 cases, neonatal 20 cases. The strategy could identify all of 32 affected fetuses. The prevalence of severe thalassemia is 32 / 5253 or 0.6% pregnancies. Among 5125 neonates of negative screening tests, no any single case of severe thalassemia was identified by neonatal testing (IEF technique). No any new cases escape from screening program.

A total of 5125 neonates evaluated for thalassemia status, either trait or diseases, is presented in Table 2 and 3

Though 8247 pregnant women were recruited, only women with had a positive screening test (either for thalassemia or HbE) needed husband testing. Women with negative for both screening tests (thalassemia test or HbE test) needed no any further test. Therefore, not all husbands were screened. Note that 8247 pregnancies were recruited, only 5125 neonates were successfully taken blood for hematological analysis with IEF (isoelectric focusing). This is due to several factors including transportation problems, failed communication between the researchers' team and physicians taking care of the parturients and several women did not give birth at the hospitals registered for the project. Therefore only 5125 pregnancies were evaluated for the effectiveness of the program and the remainders who had no final outcomes were excluded from analysis. The strategy had a sensitivity and negative predictive value of 100%. No any affected fetus was missed from the detection as presented in Table 4. The prevalence of severe thalassemia was 0.6 % (32/5243). All of the 30 affected fetuses were prenatally identified.

Table 1: Number of couples and neonates participating through the programs

Institute	No. of Woman	No. of Dx carrier	No. of Husband
Bhumibol Hospital	839	28	3
Chiang Mai University	2668	334	1692
Khonkaen University	377	124	79
Pramongkutklao Hospital	1119	74	227
Prince of Songkla University	651	123	72
Rajavithi Hospital	2593	527	814
Total	**8247**	**1210**	**2887**

Table 2: Number of couples and neonates participating through the programs

Participants	No. of Cases
Total women prospectively screened	8247
Women with positive screening test	2668
Women with positive diagnostic test for carriers	1210
The women's husbands screened	2887
Pregnancies with fetuses at risk (both of the couples were positive)	132
Prenatal diagnosis Chorionic villous sampling Amniocentesis Cordocentesis	128 *37* *6* *85*
No prenatal diagnosis	4

Table 3: Number of couples and neonates participating through the programs

	No. of cases	HbBart's disease	beta-major	bea-thal/HbE	Normal / others	Total
Prenatal diagnosis	128	10	4	18	96	32
Neonatal diagnosis	5125	0	0	0	5125	0

Table 4 Effectiveness the program in prenatal detection of severe thalassemia syndromes

Screening tests	Severe Thalassemia	No Severe Thalassemia	Total
Positive test	32	96	128
Negative test	0	5125	5125
Total	32	5221	5253

Prevalence of severe thalassemia	0.6% (32/5253)
Sensitivity (32/32)	100.0 % (95% CI: 100;100)
Specificity (5125/5221)	98.2 % (95% CI: 97.7;98.5)
Positive predictive value (32/128)	25.0 % (95% CI: 17.5;32.5)
Negative predictive value (5125/5125)	100.0 % (95% CI: 100;100)

Discussion

Thalassemia is one of the most common health problems in Southeast Asia, especially in Thailand, where the prevalence of abnormal globin genes is relatively high, resulting in high prevalence of new cases of severe thalassemia syndrome, in particular, Hb Bart's disease homozygous beta-thalassemia and beta-thalassemia /HbE disease. The ministry of Public Health had adopted a 20 year plan for control of these diseases. Prenatal control is one of the strategies proven to be highly effective in preventing new cases of severe thalassemia(8;11;21-23). The strategy includes carrier screening and detection, early prenatal diagnosis for fetuses at risk, and options of control the disease could be offered.

We have launched the strategy for prenatal control of severe thalassemia in our hospital for more than ten years. However, though we are successful with preliminary project, we have never known how many fetuses have been missed for prenatal diagnosis since all algorithms or strategies in previous reports did not evaluate the neonates of the couples with negative screening tests(8;11;23-26). Therefore, we did not know whether

these neonates had false negative test or not. To solve this problem, we need to evaluate a large number of neonates of the couples with no risk, consuming a lot expense. This is the first study aimed to evaluate the efficacy of the strategy by testing all newborns of the couples either at risk or no risk. Most strategies which have been proposed have the same principle, including identifying couple at risk, prenatal diagnosis among fetuses at risk and offering an option of termination of pregnancy in case of affected fetuses, but various tests of screening and confirmatory tests for carrier and obstetric invasive diagnoses may be varied from center to center.

Mutation types of thalassemia and hemoglobinopathies are diversified around the world. They are unique for geographical areas. For example, in southeast Asia, as well as our country, the main severe thalassemia syndrome are Hb Bart's disease, beta-thalassemia major and beta-thalassemia / HbE disease, different from other parts of the world. Each geographical area has to have its own policy for prevention and control the new cases of the severe forms. This study is an example of successful strategy in large-scale application. Note that, of 5,125 cases screened women with negative results, no any single cases of severe thalassemia in newborns were identified. However, in the real practice where laboratory quality control is not perfect, new cases of severe thalassemia may occur. In our experience, new cases of severe thalassemia may sometime be encountered in spite of prenatal control strategy. As seen in this study, the algorithm is very highly effective. This suggests that the problem in control the disease is not associated with the strategy but nature of practice without quality control.

Carrier detection in asymptomatic women can be achieved by various techniques, for example; osmotic fragility (OF) test(11;21) or means corpuscular volume (MCV) as a screening test for alpha-thalassemia-1 and beta-thalassemia carriers and using PCR or IC strip/PCR for confirmation of alpha-thalassemia-1 carriers.

Unlike all previous studies in which only detection rate was evaluated, this study also evaluated the newborns of couples at no risk. This study strongly suggests that the prenatal control strategy is highly effective in reducing the incidence of severe thalassemia syndrome among screened pregnancies, resulting in improving quality of life. Hopefully, when widely implemented, the strategy could have a great good impact on public health in geographical areas of high prevalence. We further expect that the strategy will be the basis of model for severe thalassemia control in the future.

This strategy emphasizes on effectiveness of screening and identifying carriers and couples at risk leading to prenatal diagnosis with several options. Note that the main fetal diagnostic technique for fetuses at risk of beta-thalasemia major and beta-thalassemia / HbE disease was cordocentesis with fetal blood analysis with HPLC. This is one of our limitations since cordocentesis carries a higher rate of fetal loss than amniocentesis and is performed rather late in gestation. Only few cases underwent CVS or amniocentesis with DNA analysis since DNA analysis was not available in most centers. The three invasive diagnostic techniques have their own advantages and disadvantages. A total of 40 fetuses at risk of Hb Bart's disease were effectively selected for cordocentesis by sonographic markers. Cordocentesis was done in only 10 pregnancies and all are positive and diagnosed before 22 weeks of gestation. All of the remainders with negative ultrasound markers were finally proven to be normal. Ultrasound can effectively reduce the need of cordocentesis leading to reduce procedure-related fetal loss.

Primary screening for alpha-thal-1 and beta-thal-1 carrier may be OF or MCV. The detection rate may be comparable but MCV provides fewer false positive rates (15). We prefer to use MCV because of its convenience of automated hematology analyzer. It could be performed in the same time of routine complete blood cell counts. Likewise, Hb E carrier could be screened by DCIP or CMU-E screen. We preferred CMU-E-screen since its has highest sensitivity and specificity for Hb E carriers (10;13). MCV and OF may give a false positive test in some medical disease such as iron deficiency resulting in unnecessary further work-up but they are very sensitive.

All screening tests are very high sensitive, but not perfect. Therefore, some affected fetuses may be missed from the screening program, as seen in real practice. However, the strategy presented here seems to have no false negative test, no any missing case. This might be due to too small sample size of affected fetuses to express such a low false negative rate.

Some pitfalls should be mentioned in this study. First of all, not all fetuses were followed-up until the final thalassemia status was tested, resulting in excluding the large number of pregnancies and compromising the power of test. Secondly, many pregnancies at risk did not undergo prenatal diagnosis due to several reasons, leading to exclusion from analysis. For example, the husbands of the wives with abnormal screening test did not come to be tested, some couples at risk did not receive prenatal diagnosis, and many women first presented in late pregnancies.

The risk of prenatal invasive diagnosis is another subject to be seriously considered. Though no procedure-related loss occurred in this study, this may be due to a small number of procedures done in this study. Diagnosis with ultrasound in fetuses at risk of Hb Bart's disease could obviate the need for cordocentesis. Additionally, all invasive procedures in the study presented here were performed by experienced perinatologists. However, the procedure-related fetal loss must be taken into account for the strategy risk. For example cordocentesis related fetal loss may be 1-2%(27-29). From our perspective, in widely implementation, effective screening should be effectively carried out to identify couples at risk in primary health care and invasive prenatal diagnosis should be reserved for tertiary care center.

Fetal diagnosis could be done by DNA analysis from chorionic villi or amniocytes to identify some specific mutation. Although this technique has been shown successful for prenatal diagnosis(22;23;30), it is so sophisticated, expensive and time-cosuming that could not put into practice for routine work, especially in our population which have various mutations of beta-gene(31). Therefore, we analyzed fetal blood by differenting hemoglobin types using HPLC which could differentiate types of hemoglobin even in small amount with high accuracy(32;33). However, the disadvantages of this approach are its rather late procedure and somewhat higher fetal loss when compared to amniocentesis or chorionic villi sampling. However, various techniques of DNA analysis for thalassemia are becoming widely developed, we hope that the technique of prenatal diagnosis will change to the less invasive technique,such as amniocentesis, or analysis of fetal blood in maternal serum(34;35). However, the screening test should still be simple, rapid, and inexpensive as we have been doing.

To summarize, the strategy consisted of carrier identification, prenatal diagnosis, and termination of pregnancy with affected fetuses. This strategy could prenatally identify affected fetuses with a detection rate and negative predictive value of 100%.

However, false positive was about 75%, consistent with theoretical risk of having affected fetuses 25%. The false positive results can lead to unnecessary invasive diagnosis, carrying risk of procedure-related loss. However, of fetuses at risk of Hb Bart's disease, the procedure-related loss could be avoided by serial ultrasound examination. Overall, we conclude that the strategy are very effective and are able to prenatally detect all affected fetuses with an acceptable false positive rate.

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Accuracy of Immunochromatographic Strip Test in Diagnosis of alpha-Thalassemia-1 Carrier

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Running Title: Immunochromatographic strip in diagnosis of α-thalassemia-1 carrier

Abstract

Objective: To determine the accuracy of alpha-Thal Immunochromatographic (IC) strip in diagnosis of alpha-thalassemia1 carrier among pregnant women, using PCR for alpha-thalassemia1 (SEA type) as a gold standard.

Methods: Asymptomatic pregnant women attending antenatal care clinic were recruited. Their blood samples were taken to for IC Strip Test (αThal IC strip, i+Med Laboratories Company Limited) in predicting alpha-thalassemia-1 carrier and separately sent for PCR for diagnosis of α-thalassemia1 carrier as a gold standard.

Results: Four hundred and ninety-nine pregnant women were recruited into the study at various gestational weeks. The accuracy of alpha-Thal IC strip test was relatively high as shown in table 1. Of them, 62 cases were proven to be alpha-thalassemia1 trait and all of them had the results of positive IC strip, giving a sensitivity of 100%. However, 45 pregnant women of non-α-thalassemia1 trait had positive test, giving a specificity of 89.0%.

Conclusion: This study was solid evidence for clinical application of α-thal IC strip in screening program of thalassemia to reduce the need for PCR in diagnosis of α-thalassemia1 carrier because of its very high sensitivity. The negative test reassures the non α-thalassemia1 carrier status. Moreover, due to its simplicity, convenience to use, low cost, less-time consuming, clear interpretation and no need either for equipment or expensive laboratories, it may probably be very helpful in massive screening program.

Key Words: Immunochromatographic strip, Thalassemia carrier**,** Pregnancy

Introduction

Hemoglobin (Hb) Bart's disease (homozygous a-thalassemia1) is one of the most common hematologic genetic diseases especially in South East Asia. The prevalence of α-thalassemia1 gene in our population is as high as 10-14%(1;2). Hb Bart's hydropic fetuses have never survived and their mothers often suffer from obstetric complications such as pre-eclampsia, dystocia, postpartum hemorrhage due to a large placenta, and the psychological burden for carrying a nonviable fetus to term. Each year, our department faces about 20-30 new cases of Hb Bart's disease. Therefore, this disease needs to be controlled, especially by prenatal approach(3). Recently we have a great success in control of severe thalassemia with simple way(4). However, though we have success with preliminary project, several problems need to be solved. Prenatal diagnosis of severe thalassemia is usually performed only on pregnant women who are at risk of having baby with severe thalassemia, both of the couple are carriers. To achieve the goal of prenatal control strategy, it is necessary to have a highly effective method in identifying thalassemia carriers or a couple at risk. In diagnosis of alpha-thalassemia1 carriers, various techniques of PCR (polymerase chain reaction) are most commonly used for such a purpose and often used as a gold standard(5). However, though PCR is associated with reliable accuracy and precision. It has some limitations, preventing them from being widely used. For example, PCR is expensive, not widely available especially in the rural areas, need of sophisticated laboratory and experienced or well-trained performers. Therefore, new cheaper techniques to identify α-thal-1 carrier need to be sought for. We recently developed a new test, Alpha Thalassemia immunochromatographic strip test (αThal IC strip, i+Med Laboratories Company Limited) for such a purpose(6). This is a lateral flow chromatographic immunoassay to determine Hb Bart's in red blood cell hemolysates, which is usually present in small amount in red blood cells of the carriers of alpha thalassemia1. This new technique is easy to perform, inexpensive, quick and no equipment necessary and this is the world's first alpha thalassemia immunochromatographic strip test. Based on pilot test, alpha-Thal IC strip may have sensitivity as high as 100% and specificity of 98%. However, alpha-Thal IC strip test has never been tested in clinical application especially in pregnant women. Because of its simplicity, low cost, no equipment needed, if its accuracy in diagnosis of alpha-thalassemia 1 is comparable to PCR technique, it must be very helpful in large-scaled screening system. Therefore, this new alpha-Thal IC strip should first be tested for its accuracy together with standard technique to determine its accuracy before being used as a single diagnostic test or screening test for PCR. The objective of this study was to determine the accuracy of alpha-Thal IC strip in diagnosis of alpha-thalassemia1 carrier among pregnant women, using PCR for alpha-thalassemia1 (SEA type) as a gold standard.

Materials and Methods

The population for the study is pregnant women attending antenatal care clinic at first visit at Maharaj Nakorn Chiang Mai Hospital, between October, 2007 and May, 2008. These pregnant women were routinely screened for thalassemia carriers, as a part of prenatal control of severe thalassemia program(7), which has been established in our hospital for fifteen years. All pregnant women would be invited into the study. Blood sample of 2 ml will be taken and sent separately to different laboratories for the following tests: 1) alpha-Thal IC strip test, and 2) DNA analysis for alpha-thalassemia1 gene (α-thal-1; SEA type) by polymerase chain reaction or PCR technique. The two tests were separately and blindly performed by the two performers at different laboratories.

The definitions used in this study are as follows: **1) α-thalassemia-1 carrier** was an individual, who had deletion of both loci from one chromosome (- -/αα) resulting in clinically in α-thalassemia 1 trait, which is characterized by miminal hypochromic microcytic anemia or no anemia, usually not associated with clinical abnormality and often goes unrecognized. **2) alpha-Thal IC strip Test**: The test Alpha-Thal IC Strip (commercial set; i+LAB aThal IC strip Test) is a qualitative, lateral flow immunoassay for the screening of minimal amount of Hb Bart's in the specimen, **3) Polymerase chain reaction (PCR) for α-thal-1 (SEA type)**: PCR in this project used the technique which was modified from Chang's method(5;8) by changing the primer specific for α-thal-1 trait. In normal subject, PCR product will consist of only 314 base pair type, but there are PCR product of 314 and 188 base pair types in blood sample from alpha-thalassemia1 trait.

Priciple of immunochromatographic strip test (IC strip)

The immunochromatographic (IC) strip is a qualitative, lateral flow immunoassay for the screening of various types of soluble protein antigens. In our case, we developed an IC strip test for determination of Hb Bart's in blood sample.

The IC strip composes of 1) sample pad for carrying sample, 2) gold conjugate pad which contain colloidal gold labeled anti Hb Bart's monoclonal antibodies, 3) capture line contains anti Hb Bart's monoclonal antibody and this antibody is fixed on the membrane, 4) control line contains anti mouse immunoglobulin antibody and this antibody is also fixed on the membrane and 5) absorbing pad (Figure 1).

As the blood sample (hemolysate) diffuses through the absorbent test strip, in the presence of Hb Bart's in the blood sample, the colloidal gold labeled anti Hb Bart's monoclonal antibodies (in the gold conjugate pad) bind to the Hb Bart's in the specimen forming an antibody-antigen complex. This complex then binds to the anti Hb Bart's antibody in the capture (test) line and expresses a purple-red band. The absence of a colored band in the test region indicates a negative result. The reaction mixture continues flowing through the absorbent device past the capture and control lines. Unbound conjugate binds to the anti-mouse immunoglobulin antibody in the control line, producing a pink color band, demonstrating that the reagents and test strip are functioning correctly. Figure 2 shows conjugate actions in immunochromatographic strip test for negative and positive reading.

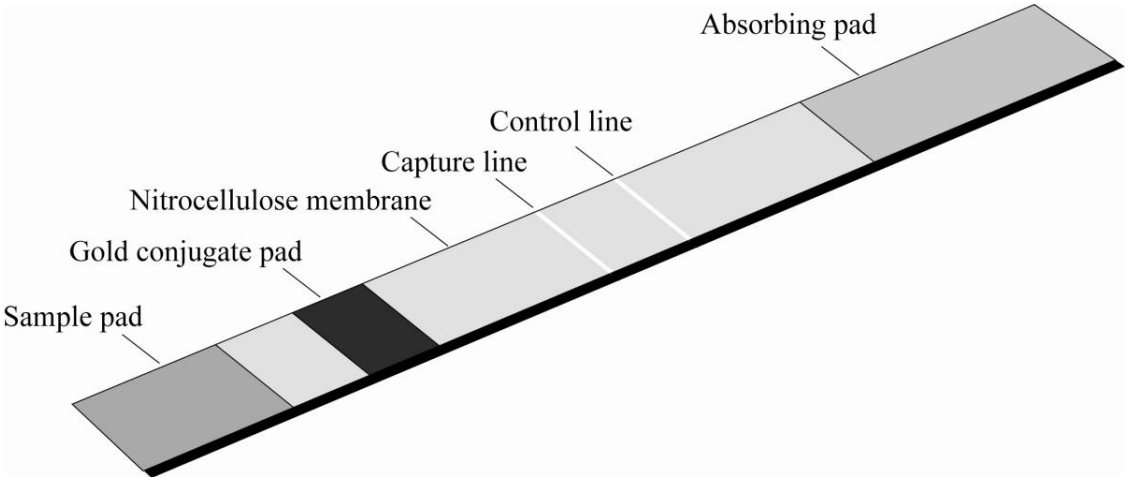

Figure 1 Outline positioning of immunochromatographic test strip components comprise sample pad, conjugate pad, membrane, capture line, control line and absorbing pad.

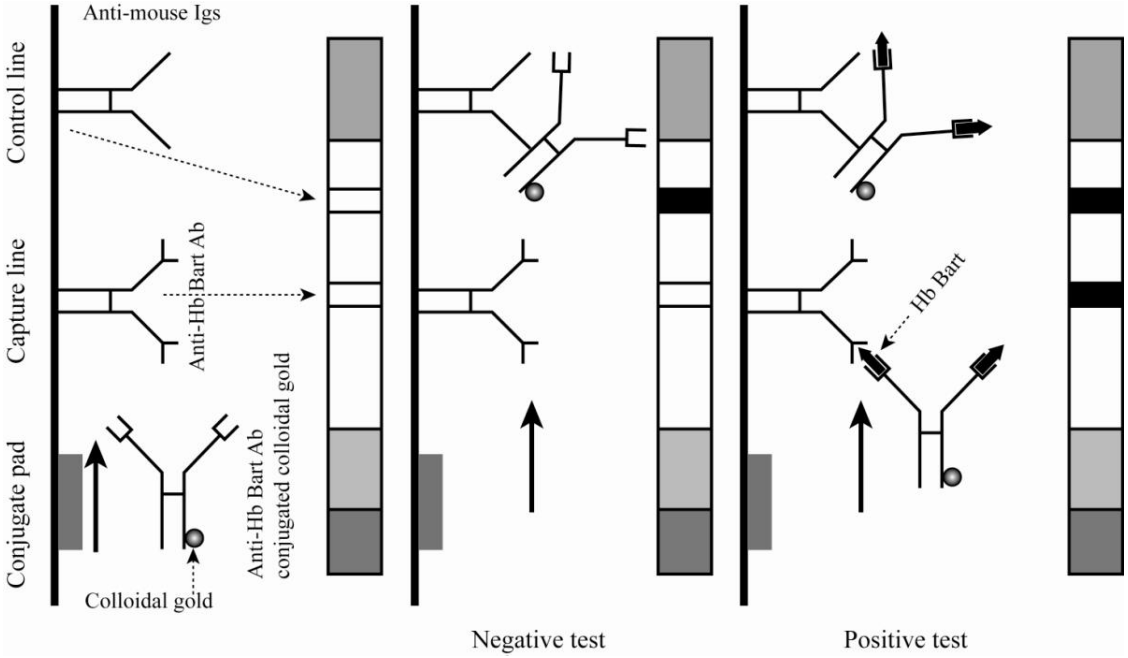

Figure 2 Diagram shows conjugate actions in immunochromatographic strip test for negative and positive reading.

Screening of α-thalassemia 1 by alpha-Thal Immunochromatographic (IC) strip test: 100 µl of EDTA-blood sample and 100 µl of RBC lysis buffer (1% TritonX-100 in distilled water) was added into a 96-well plate and mixed thoroughly. The IC strip was then immersed vertically in the hemolzyed blood for 2-5 minutes with the arrows on the strip pointing down. After that, the strip was removed and washed until the background was clear using washing buffer (0.05% Tween-PBS) and a wash bottle. The test result was then be read visually. For a positive result, two pink color bands appeared, one at the capture line zone and one the control line zone. For a negative result, only one pink color band was observed in the control line zone.

Main outcome measure was accuracy of alpha Thal IC strip in predicting alpha-thalassemia1 carriers which was calculated and presented as sensitivity, specificity, positive predictive value and negative predictive value.

This study was approved by the Research Ethics Committee, Faculty of Medicine, Chiang Mai University (Study code: 07SEP051347)

Results

Four hundred and ninety-nine pregnant women were recruited into the study at various gestational weeks. The accuracy of alpha-Thal IC strip test was relatively high as shown in table 1. Of them, 62 cases were alpha-thalassemia1 trait and all of them gave the results of positive IC strip, sensitivity of 100%. However, 45 pregnant women who were proven to be non-a-thalassemia1 trait gave positive test (false positive rate of 11%). The alpha-Thal IC strip test had specificity of 89.0%.

Table 1: The accuracy of alpha-Thal IC Strip in predicting a-thalassemia1 carrier among total obstetric population, using PCR as a gold standard

IC-Strip	**PCR**		**Total**
	Pos	Neg	
Positive	62	48	110
Negative	0	389	389
Total	62	437	499

Sensitivity: 100% (62/62)
Specificity: 89.0% (389/437)
Positive predictive accuracy: 56.4% (62/110)
Negative predictive accuracy: 100.0% (389/389)
Accuracy 90.0% (451/499) 95% CI: (87.4-92.8%)
Prevalence 12.4% (62/499) 95% CI: (12.4-12.4%)

Discussion

This is the first report on evaluation of the accuracy of IC strip in detection of Hb Bart's in blood samples, and it suggests that IC strip has a potential role in clinical application for identifying alpha-thalassemia1 trait which plays an important role in prenatal control of severe thalassemia syndrome. IC strip test is developed to determine small amount of Hb Bart's qualitatively. Therefore any conditions or diseases with the presence of Hb Bart's will yield a positive test. Minimal concentration of Hb Bart's in red blood cells is rather specific for alpha-thalassemia1 trait, though other rare conditions can also be positive. If this test is specific for alpha-thalassemia1 trait, it will be extremely useful in clinical application to screen or diagnose for alpha-thalassemia1 carrier.

In diagnosis of alpha-thalassemia1 carriers, various techniques of PCR (polymerase chain reaction) are most commonly used for such a purpose and often used as a gold standard(5;7;9-12). However, though PCR is associated with reliable accuracy and precision, it has some limitations, preventing them from being widely used. For example, PCR technique is expensive, not widely available especially in the rural areas, need of sophisticated laboratory and experienced or well-trained performers. Therefore, a cheaper new test, alpha-Thal IC strip test has been developed for this purpose (6). However, alpha-Thal IC strip test has never been tested in clinical application especially in pregnant women. Therefore, this new IC strip should first be tested for its accuracy together with standard technique to evaluate its accuracy before clinical application.

Our results indicated that the IC strip test is very sensitive. All cases with a-thalassemia1 trait gave a positive result. However, it is not highly specific since several cases other than alpha-thalassemia1 trait could have positive reactivity. Not all positive tests were a-thalassemia1 trait. This is due to the fact that some rare conditions may have small amount of Hb Bart's in circulation such as Hb H (α thal 1 / α thal 2), Hb H-CS (α thal 1 / Hb Constant Spring), AE Bart's disease (Hb H disease + HbE trait) and homozygous alpha-thalassemia2 (6). Apparently, IC strips could not replace PCR as a diagnostic test since it has some false positive tests. However, it may be used as screening test for PCR. Due to very high negative predictive value, negative IC strip may obviate the need of PCR. With this approach, PCR could be avoided in more than two-thirds of cases with positive screening test. Its perfect sensitivity and negative predictive value make this test very valuable in identifying cases for further confirmation with PCR and the reduction in expense in prenatal control would be substantial.

PCR we used as gold standard in this study was specific only for SEA type α-thalassemia1 trait. We don't test for other variants of mutations. Since this is the only type we are concerned. Therefore, it is possible that a-thalassemia1 carrier of other mutation can be missed and this will explain false positive of unknown in some tests. On the other hand, IC strip may also be helpful in other mutations of a-thalassemia1 since it tests the presence of Hb Bart's in red blood cells regardless mutation types.

The strength of this study was associated with high reliability of laboratory testing since the technicians performed IC strips and PCR were blinded to each other. Moreover, the sample size was adequate. The result from this study was solid evidence for clinical application of alpha-thal IC strip in screening program of thalassemia to reduce the need for PCR in diagnosis of a-thalassemia1 carrier. The negative test reliably excludes alpha-thalassemia1 carrier status whereas no any single case of alpha-thalaseemia1 carrier will

be missed. Moreover, due to its simplicity, convenience to use, low cost, less-time consuming, clear interpretation and no need either for equipment or expensive laboratories, it may probably be very helpful in massive screening program.

In conclusion, though, alpha-thal IC could not replace PCR. The result from this study was solid evidence for clinical application of alpha-thal IC strip in screening program of thalassemia to reduce the need for PCR in diagnosis of a-thalassemia1 carrier especially in the case of negative test for alpha-thal IC because of its very high negative predictive value. The negative test reassures the non a-thalassemia1 carrier status. Moreover, due to its simplicity, convenience to use, low cost, less-time consuming, clear interpretation and no need either for equipment or expensive laboratories, it may probably be very helpful in massive screening program. Alpha-thal IC strip has potential to play an important role to reduce the cost of prenatal control strategy. We believe that alpha-thal IC strip can contribute to the strategy to be the cheapest prenatal control program ever done.

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Splenic Circumference at Midpregnancy as a Predictor of Hemoglobin Bart's Disease among Fetuses at Risk

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Splenic Circumference at Midpregnancy as a Predictor of Hemoglobin Bart's Disease among Fetuses at Risk

Abstract

Objective: To determine the accuracy of splenic circumference in predicting hemoglobin (Hb) Bart's disease among fetuses at risk at mid-pregnancy

Materials & Methods: Singleton pregnancies with fetal risk of Hb Bart's disease were enrolled to the study at 18-22 weeks of gestation. All underwent splenic circumference measurement before cordocentesis for fetal blood analysis. The final diagnosis used as a gold standard was based on fetal hemoglobin typing using high performance liquid chromatography (HPLC).

Results: Of pregnancies recruited, the prevalence of Hb Bart's disease was 26.1% (87/334 fetuses). Twenty-four fetuses (27.6%) had some degree of hydopic changes. Notably, of these 24, 22 showed splenomegaly as well. When hydropic fetuses were excluded, the sensitivity, specificity, positive predictive value and negative predictive value of splenic circumference in identifying affected fetuses was 68.3%, 83.0%, 50.6% and 91.1% respectively.

Conclusion: Splenic circumference measurement at mid-pregnancy may be helpful in distinguishing affected fetuses from those unaffected. Among couples at risk with normal splenic size, the risk of having an affected child is much lower whereas the enlarged spleen places the pregnancy to be at a higher risk. This information may help the couples make decision on invasive diagnosis or non-invasive approach.

Introduction

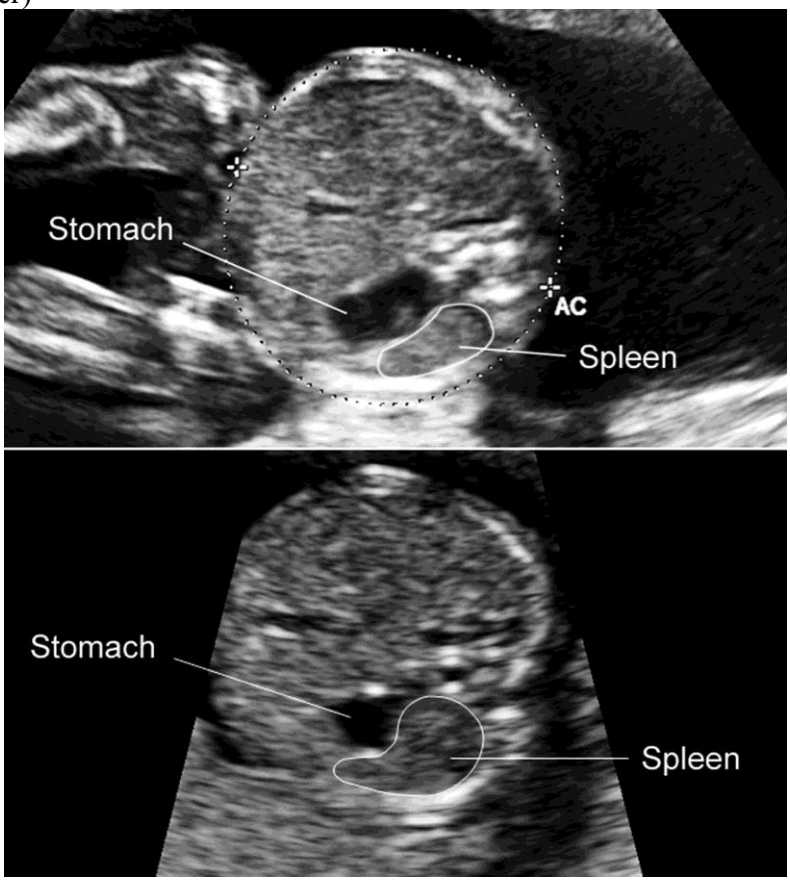
Hb Bart's disease (homozygous a-thalassemia-1) is the most common cause of hydrops fetalis in South East Asia(1-4). With population migrations during the past decade, this disease is now seen in increasing number in other parts of the world. The affected fetuses are inevitably either stillborn or die shortly after birth(4). Moreover, serious maternal complications are frequently seen in pregnancies with affected fetuses, including severe preeclampsia, dystocia, postpartum hemorrhage due to large placenta and the psychological burden for carrying a nonviable fetus to term. It is therefore essential that prenatal diagnosis and early termination of the pregnancy be recommended. Currently, several sonographic markers may be helpful in predicting fetal Hb Bart's disease before hydropic changes occur(5;6). However, the effectiveness of splenic size measurement has never been evaluated. Splenic enlargement associated with extramedullary erythropoiesis secondary to fetal anemia due to either Hb Bart's disease or Rh alloimmunization has already been known. Some authors demonstrated that the splenic size could identify affected pregnancies with high sensitivity and specificity but they included only patients at late pregnancies and focused on fetuses at risk of Rh alloimmunization(7;8). Although the classic hydropic changes due to Hb Bart's disease is likely to occur after 20 weeks(9), our extensive experience of prenatal diagnosis by fetal blood analysis at 16-22 weeks suggests that fetal splenic size measurement may be useful in differentiating normal fetuses from affected fetuses. We hypothesize that splenic circumference measurement may be useful in identifying fetal Hb Bart's disease among fetuses at risk. The objective of this study was to evaluate the efficacy of fetal sonographic splenic size at midpregnancy in predicting fetal Hb Bart's disease before the appearance of classic ultrasound findings of hydrops fetalis.

Materials and Methods

This study was conducted at Maharaj Nakorn Chiang Mai Hospital, Department of Obstetrics and Gynecology, Faculty of Medicine, Chiang Mai University with an approval of the research ethical committee. Pregnancies were recruited to the study with written informed consent and met the following inclusion criteria: 1) fetuses at risk of Hb Bart's disease (both of the couples were a carrier), 2) gestational age of 18-22 weeks, based on a reliable last menstrual period or sonographic biometric measurements in the first half of pregnancy, 3) singleton pregnancies, 4) fetal diagnosis of Hb Bart's disease on the basis of cordocentesis and fetal blood analysis with high-performance liquid chromatography (HPLC). Before cordocentesis, standard ultrasound examination was performed for fetal biometry and anomaly screening as well as splenic circumference measurement. Pregnancies with fetal chromosome abnormalities or anomalies other than Hb Bart's disease were excluded from analysis. All ultrasound examinations were performed by the authors using transabdominal real-time scanners with Aloka SSD alpha-10 model (Aloka Co, Ltd, Tokyo, Japan) or GE Voluson E8 (GE Healthcare, USA), having a transducer frequency of 3.5 MHz were performed by the authors. The sonographic images and measurements were automatically, digitally recorded for subsequent analysis. On the splenic measurement, the ultrasound transducer was oriented to obtain the transverse view of the fetal upper abdomen and then the fetal spleen was identified as a crescent-shaped or triangular structure with homogeneous echo density, nearly or slightly echogenic than the liver, posterior to the fluid-filled stomach and lateral to the left adrenal gland. The splenic circumference was measured by the manual trace method

(Figure 1). The three best measurements were digitally recorded in the computerized record form and subsequently averaged for analysis. Splenomegaly or abnormally enlarged splenic size was defined as a splenic circumference of larger than 95th percentile based on normal reference ranges derived from our own population(10). Baseline demographic and obstetric data of the pregnant women were also recorded digitally at the same time. The pregnant women were treated as standard antenatal care. The stored data were analyzed for the efficacy of fetal splenic circumference in predicting fetal Hb Bart's diseases, using the statistical package for the social sciences (SPSS) version 17.0 (Chicago, IL). The measurement outcomes were presented as sensitivity, specificity, positive and negative predictive value.

Figure 1: Measurement of the fetal spleen in a normal fetus at 20 weeks of gestation (upper) and a fetus with hemoglobin Bart's disease at 19 weeks of gestation (lower)



Results

During the study period (June 2007 - February 2010), 355 pregnancies at risk for having fetuses with Hb Bart disease were recruited to the study. Three hundred and thirty-four were sonographically evaluated at 18-22 weeks and underwent cordocentesis with available final diagnosis. Of these, 87 were finally proved to be affected by Hb Bart disease, and the remainders were unaffected. Mean gestational age ($\pm$SD) was 19.8$\pm$1.2 weeks and 20.11$\pm$1.3 weeks for normal and affected pregnancies, respectively (Student's t test; $p > 0.05$). The mean maternal age was 27.0 years, no difference between the two groups. Of them, 42.5% were nulliparous and

57.5% were multiparous. No serious maternal complications, such as pregnancy-induced hypertension, were noted at midpregnancy. The mean (±SD) splenic circumference in normal fetuses and fetuses with Hb Bart's disease were 4.3±1.5 and 6.7±1.5 cms, respectively, (Student's t test; $p < 0.001$), as presented in Figure 2.

The sensitivity and specificity of splenic circumference in differentiating fetuses with Hb Bart disease from the unaffected ones are summarized in Table 1. Among all fetuses at risk, the splenic circumference had sensitivity of 70.1% and a specificity of 83.0%. Twenty-four fetuses (27.6%) had some degree of hydops fetalis, such as minimal ascites or pleural effusion. Notably, of these 24, 22 showed splenomegaly as well.

When hydropic fetuses were excluded, the sensitivity, specificity, positive predictive value and negative predictive value of splenic circumference in identifying affected fetuses was 68.3%, 83.0%, 50.6% and 91.1% respectively.

Figure 2: Scattergram of the measurement values of fetal splenic circumference of normal fetuses and fetuses with hemoglobin Bart's disease

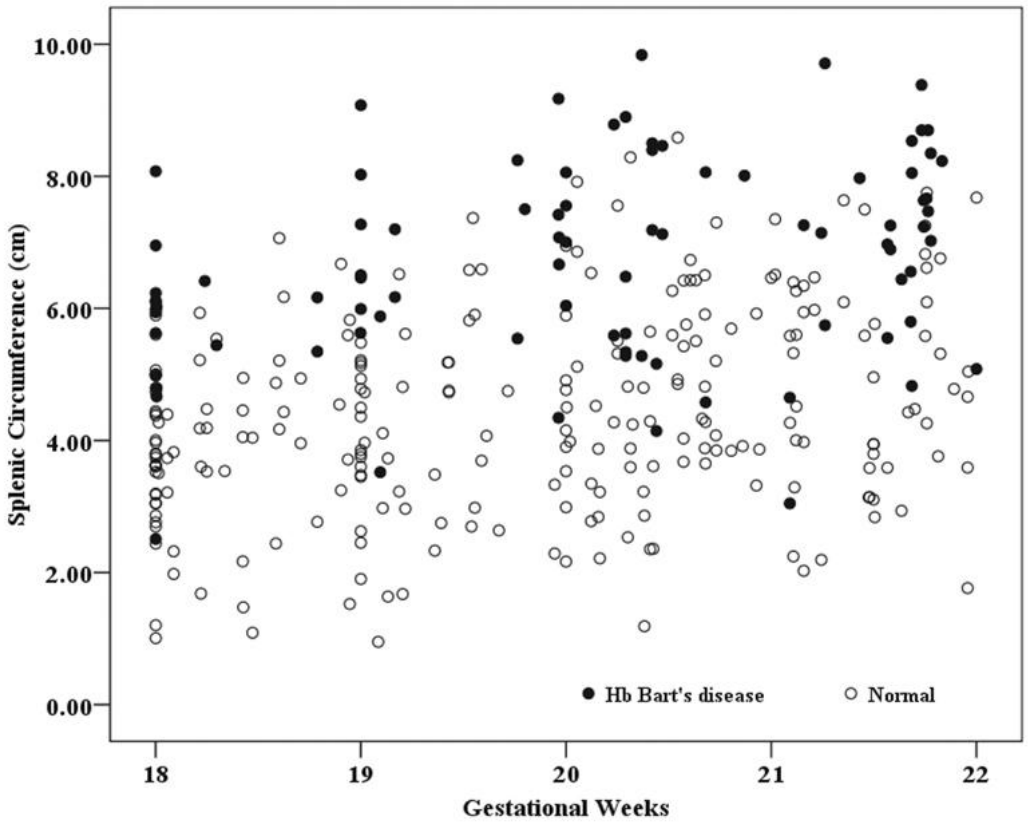


Table 1 Diagnostic indices of splenic circumference in predicting Hb Bart's disease

Splenic circumference	Hb Bart's disease	No Hb Bart's disease	Total
Positive	61	42	103
Negative	26	205	231
Total	87	247	334

Prevalence: 26.1% (87/334)
Sensitivity: 70.1% (61/87) (95% CI: 60.5-79.7%)
Specificity: 83.0% (205/247) (95% CI: 78.3-87.7%)
Positive predictive value: 59.2% (61/103) (95% CI: 49.7-68.7%)
Negative predictive accuracy: 88.7% (205/231) (95% CI: 82.6-94.8%)

Table 2 Diagnostic indices of splenic circumference in predicting Hb Bart's disease

Splenic circumference	Hb Bart's disease	No Hb Bart's disease	Total
Positive	43	42	85
Negative	20	205	225
Total	63	247	310

Prevalence: 20.3% (63/310)
Sensitivity: 68.3% (43/63) (95% CI: 56.8-79.7%)
Specificity: 83.0% (205/247) (95% CI: 78.3-87.7%)
Positive predictive value: 50.6% (43/85) (95% CI: 40.0-61.2%)
Negative predictive accuracy: 91.1% (205/225) (95% CI: 85.1-97.2%)

Discussion

As already known, fetal anemia secondary to various causes usually leads to extramedullary erythropoesis resulting in splenic enlargement. Therefore, theoretically, splenic size assessment may probably be effective in identifying fetal anemia. Previous studies showed that the splenic size was highly effective in identifying fetuses affected by Rh alloimmunization(7;8). Oepkes et al. (8) found that splenic circumference was an excellent predictor of severe anemia (hemoglobin deficit > 5 SD from normal mean) in 44 of 47 cases, a positive predictive value of 94%. However, though consistent with those in previous studies, our results indicated that splenic size measurement was not as excellent predictor of anemia as seen in Oepkes's and Bahado-Singh's report which was studied in late gestation. This may be due to the fact that splenic size at midpregnancy (18-22 weeks of gestation) may be not as sensitive in differentiating affected from unaffected fetuses as in later pregnancy. At mid-pregnancy, most fetuses with Hb Bart's disease do not develop hydropic changes yet and fetal anemia may not so severe at this stage of pregnancy. Additionally, splenic circumference values are signficantly overlapped among affected and unaffected fetuses. However, we believe that in advanced gestational age when the fetal anemia becomes more severe, splenic circumference is likely to have higher sensitivity and specificity in predicting anemia.

Among fetuses at risk, at mid-pregnancy, splenic circumference can distinguish fetuses with Hb Bart's disease with sensitivity of 70% and specificity of 83%. This suggests that splenic size assessment may not be a good screening tool in indentifying affected fetuses when compared to cardiothoracic ratio(5;11) or middle cerebral artery peak systolic velocity(12). However, this may be useful as an adjunct with other sonographic markers in identifying fetal anemia especially in fetuses at risk but it should not be used alone. Though splenic circumference may be not a perfect tool in predicting Hb Bart's disease, it is simple and is likely to be useful when combined with other parameters. It is important to emphasize that normal splenic circumference at 18 to 22 weeks reduces the probability that a fetus is affected by the disease and may obviate the need for invasive procedures to confirm normality, especially in case of no other sonomarkers such as cardiomegaly of placentomegaly, although follow-up sonographic examinations may be required.

The strength of this study include 1) the largest sample size of pregnancy at risk ever done, 2) a prospective nature in which the examiners did not know the diagnosis at the time of ultrasound examination, and finally 3) the normative reference ranges of splenic size were based on our own data, derived from the same population as the study group. The weakness of this study is that it may have included bias in the measurement of splenic circumference. The measurement was not a completely blind method since the examiner could visualize the morphology of the fetuses, especially cardiomegaly on sonographic imaging. Therefore, signs of early hydropic change in affected fetuses, such as cardiomegaly or placentomegaly, could have been visible in some cases and the diagnosis of Hb Bart's in such fetuses might have been anticipated. Additionally, interobserver variation among the sonologists was not tested.

In conclusion, splenic size assessment at mid-pregnancy can be helpful in differentiating affected fetuses from those unaffected, although not absolutely. Among couples at risk with normal splenic size, the risk of having an affected child is much lower whereas the enlarged spleen places the pregnancy to be at a higher risk. This information may help the couples make decision on invasive diagnosis or non-invasive approach.

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Reference ranges of fetal aortic and pulmonary valve diameter derived by STIC from 14 to 40 weeks of gestation

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Objective To develop reference ranges of fetal aortic and pulmonary valve diameter derived from volume datasets of spatio-temporal image correlation (STIC).

Methods A cross-sectional study was undertaken on low-risk pregnancies with well-established data from 14 to 40 weeks. Volume datasets of STIC were acquired for subsequent off-line analysis. Aortic and pulmonary valve diameters were measured in STIC multiplanar view using 4D-View version 9. Normal *Z* scores and centile reference ranges were constructed from these measurements against gestational age (GA) and biparietal diameter (BPD) as independent variables, using regression models for both mean and SD.

Results A total of 606 volume datasets were successfully measured. Normal reference ranges for predicting mean values and SD of aortic and pulmonary valve diameter were constructed based on best-fit equations (linear function) as follows: mean aortic diameter (mm) was modeled as a function of GA (weeks) and BPD (mm) as $-2.4838 + 0.2702 \times$ GA, (SD $= 0.1482 + 0.0156 \times$ GA) and $-1.5952 + 0.0989 \times$ BPD (SD $= 0.1672 + 0.00572 \times$ BPD). Mean pulmonary diameter was modeled as $-2.5924 + 0.2935 \times$ GA (SD $= 0.2317 + 0.01524 \times$ GA) and $-1.6830 + 0.1083 \times$ BPD (SD $= 0.1971 + 0.0059 \times$ BPD).

Conclusion We have provided nomograms and *Z* scores of fetal aortic and pulmonary valve diameters. These reference ranges may be a useful tool in the assessment of fetal cardiac abnormalities.

KEY WORDS: aortic valve; prenatal; pulmonary valve; reference range; spatio-temporal image correlation (STIC); 4D-ultrasound; *Z* scores

INTRODUCTION

Measurement of the size of the heart structures has been a part of the fetal echocardiography to make diagnosis and evaluate prognosis of the disease (Rychik *et al*., 2004; Anon, 2006). In diagnosis of anomalies of the great vessels, such as aortic stenosis, coarctation of the aorta, pulmonary atresia, and tetralogy of Fallot, size of the aortic and pulmonary valves need to be accurately measured and compared with the normal values. Previous publications have reported fetal aortic and pulmonary valves' reference ranges in percentile reference charts (DeVore *et al*., 1985; Sharland and Allan, 1992; Tan *et al*., 1992; Shapiro *et al*., 1998). Though several reports on reference ranges of aortic and pulmonary valves have been published, only the studies by Schneider *et al*. (2005) and Lee *et al*. (2010) provide *Z* scores as a quantitative assessment for clinical practice, allowing for a more precise evaluation of complex cardiac defects that severely alter cardiac dimensions. Indeed, Z scores allow the examiner to quantify cardiac dimensions and are not limited by traditional confidence intervals, as was the case in previous publications (DeVore *et al*., 1985; Sharland and Allan, 1992; Tan *et al*., 1992; Shapiro *et al*., 1998). However, Schneider's study suffers from a small sample size (Lee *et al*., 2010), whereas the large study reported by Lee *et al*. (2010) is based on a retrospective assessment of their database. Additionally, the new *Z* score reference ranges in such studies (Schneider *et al*., 2005; Lee *et al*., 2010) were derived from two-dimensional (2D) fetal echocardiography, in which it is relatively hard to get precise planes for cardiac studies compared with the four-dimensional (4D) ultrasound with spatio-temporal image correlation (STIC). With STIC, the interested structures are displayed in all dimensions allowing more accurate measurements (Chaoui and Heling, 2005). Therefore, we conducted this study to develop the Z-score and centile reference ranges for fetal aortic and pulmonary valves diameter derived from 4D-STIC with adequate sample size.

MATERIALS AND METHODS

This cross-sectional descriptive study was undertaken between 1 September 2007 and 31 October 2009 at Maharaj Nakorn Chiang Mai Hospital, Chiang Mai University, Thailand. Low-risk pregnant women were recruited from our prenatal care clinic with written informed consents. The inclusion criteria were as follows: (1) gestational age (GA) between 14 and 40 weeks, (2) no known medical and obstetric

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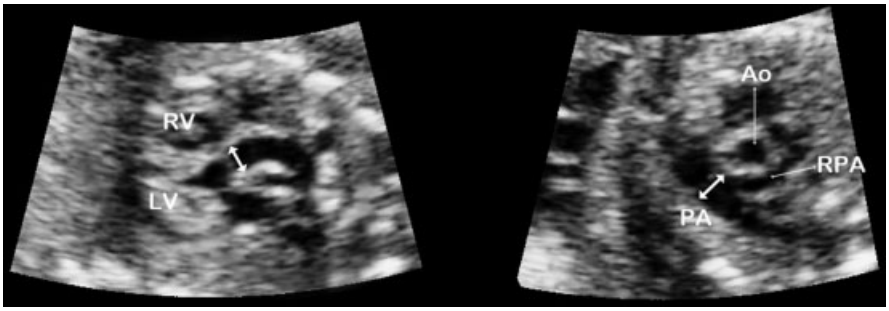

Figure 1—Examples of fetal aortic (left) and pulmonary (right) valve diameter displayed and measured in multiplanar views of STIC. (RV: right ventricle, LV: left ventricle, Ao: aorta, PA: pulmonary artery, RPA: right pulmonary artery)

Table 1—Aortic and pulmonary valve diameter (mm) as a function of gestational age (week) (*n* represents the sample size for each gestational weeks)

		Aortic valve diameter (mm)							Pulmonary valve diameter (mm)						
GA (week)	*n*	2.5th	5th	10th	50th	90th	95th	97.5th	2.5th	5th	10th	50th	90th	95th	97.5th
14	15	0.6	0.7	0.8	1.3	1.8	1.9	2.0	0.6	0.8	0.9	1.5	2.1	2.2	2.4
15	15	0.8	0.9	1.1	1.6	2.1	2.2	2.3	0.9	1.1	1.2	1.8	2.4	2.6	2.7
16	17	1.1	1.2	1.3	1.8	2.3	2.5	2.6	1.2	1.3	1.5	2.1	2.7	2.9	3.0
17	18	1.3	1.4	1.6	2.1	2.6	2.8	2.9	1.4	1.6	1.8	2.4	3.0	3.2	3.4
18	19	1.5	1.7	1.8	2.4	2.9	3.1	3.2	1.7	1.9	2.0	2.7	3.3	3.5	3.7
19	18	1.8	1.9	2.1	2.7	3.2	3.4	3.5	2.0	2.1	2.3	3.0	3.7	3.8	4.0
20	16	2.0	2.2	2.3	2.9	3.5	3.7	3.8	2.2	2.4	2.6	3.3	4.0	4.2	4.3
21	22	2.3	2.4	2.6	3.2	3.8	4.0	4.1	2.5	2.7	2.9	3.6	4.3	4.5	4.7
22	26	2.5	2.7	2.8	3.5	4.1	4.3	4.4	2.8	2.9	3.1	3.9	4.6	4.8	5.0
23	27	2.7	2.9	3.1	3.7	4.4	4.6	4.7	3.0	3.2	3.4	4.2	4.9	5.1	5.3
24	33	3.0	3.1	3.3	4.0	4.7	4.9	5.0	3.3	3.5	3.7	4.5	5.2	5.4	5.6
25	33	3.2	3.4	3.6	4.3	5.0	5.2	5.3	3.5	3.7	4.0	4.7	5.5	5.8	5.9
26	25	3.5	3.6	3.8	4.5	5.3	5.5	5.6	3.8	4.0	4.2	5.0	5.8	6.1	6.3
27	30	3.7	3.9	4.1	4.8	5.5	5.7	5.9	4.1	4.3	4.5	5.3	6.2	6.4	6.6
28	26	3.9	4.1	4.3	5.1	5.8	6.0	6.2	4.3	4.5	4.8	5.6	6.5	6.7	6.9
29	23	4.2	4.4	4.6	5.4	6.1	6.3	6.5	4.6	4.8	5.1	5.9	6.8	7.0	7.2
30	29	4.4	4.6	4.8	5.6	6.4	6.6	6.8	4.9	5.1	5.3	6.2	7.1	7.3	7.6
31	35	4.7	4.9	5.1	5.9	6.7	6.9	7.1	5.1	5.3	5.6	6.5	7.4	7.7	7.9
32	26	4.9	5.1	5.3	6.2	7.0	7.2	7.4	5.4	5.6	5.9	6.8	7.7	8.0	8.2
33	27	5.1	5.3	5.6	6.4	7.3	7.5	7.7	5.7	5.9	6.2	7.1	8.0	8.3	8.5
34	22	5.4	5.6	5.8	6.7	7.6	7.8	8.0	5.9	6.2	6.4	7.4	8.3	8.6	8.9
35	28	5.6	5.8	6.1	7.0	7.9	8.1	8.3	6.2	6.4	6.7	7.7	8.7	8.9	9.2
36	24	5.9	6.1	6.3	7.2	8.2	8.4	8.6	6.4	6.7	7.0	8.0	9.0	9.3	9.5
37	22	6.1	6.3	6.6	7.5	8.4	8.7	8.9	6.7	7.0	7.2	8.3	9.3	9.6	9.8
38	14	6.3	6.6	6.8	7.8	8.7	9.0	9.2	7.0	7.2	7.5	8.6	9.6	9.9	10.2
39	9	6.6	6.8	7.1	8.1	9.0	9.3	9.5	7.2	7.5	7.8	8.9	9.9	10.2	10.5
40	7	6.8	7.1	7.3	8.3	9.3	9.6	9.8	7.5	7.8	8.1	9.1	10.2	10.5	10.8

complications, and (3) reliable GA based on regular menstrual cycle and certain last menstrual period consistent with sonographic fetal biometry in the first half of pregnancy. The exclusion criteria were (1) multifetal pregnancy, (2) fetal anomalies, (3) abnormal fetal growth (either fetal growth restriction or macrosomia), and (4) satisfactory 4D-STIC volume datasets not obtainable.

4D-STIC volume datasets were obtained using real-time equipment with transabdominal 2 to 5 MHz curvilinear transducers, Voluson E8 (GE Healthcare, USA). In each examination, a standard ultrasound examination, including fetal biometry and anatomical survey, was first performed. 4D volume datasets of the fetal heart were acquired with transverse sweeps through the fetal chest in which the regions of interest (ROI) were properly selected including either the apical or subcostal heart views. Acquisition time ranged from 7.5 to 15 s and the angle of acquisition ranged between 20° and 40° depending on fetal motion and GA. All of the volume datasets were stored in the ultrasound machine hard disk for subsequent off-line analysis using a 4D view version 9.0 (GE Medical Systems, Zipf, Austria). The systematic approach for visualization of the great vessels in multiplanar display was performed off-line to identify aortic and pulmonary valve as described by Goncalves *et al*. (2006), or DeVore *et al*. (2004), as shown in Figure 1. The greatest dimensions of the

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Table 2—Aortic valve diameter (mm) as a function of biparietal diameter (mm)

BPD	2.5th	5th	10th	50th	90th	95th	97.5th	BPD	2.5th	5th	10th	50th	90th	95th	97.5th
25	0.3	0.4	0.5	0.9	1.3	1.4	1.5	61	3.4	3.6	3.8	4.4	5.1	5.3	5.4
26	0.4	0.5	0.6	1.0	1.4	1.5	1.6	62	3.5	3.7	3.9	4.5	5.2	5.4	5.6
27	0.4	0.5	0.7	1.1	1.5	1.6	1.7	63	3.6	3.8	4.0	4.6	5.3	5.5	5.7
28	0.5	0.6	0.8	1.2	1.6	1.7	1.8	64	3.7	3.9	4.1	4.7	5.4	5.6	5.8
29	0.6	0.7	0.8	1.3	1.7	1.8	1.9	65	3.8	3.9	4.1	4.8	5.5	5.7	5.9
30	0.7	0.8	0.9	1.4	1.8	1.9	2.0	66	3.9	4.0	4.2	4.9	5.6	5.8	6.0
31	0.8	0.9	1.0	1.5	1.9	2.0	2.1	67	4.0	4.1	4.3	5.0	5.7	5.9	6.1
32	0.9	1.0	1.1	1.6	2.0	2.1	2.3	68	4.0	4.2	4.4	5.1	5.8	6.0	6.2
33	1.0	1.1	1.2	1.7	2.1	2.3	2.4	69	4.1	4.3	4.5	5.2	5.9	6.2	6.3
34	1.1	1.2	1.3	1.8	2.2	2.4	2.5	70	4.2	4.4	4.6	5.3	6.1	6.3	6.4
35	1.1	1.3	1.4	1.9	2.3	2.5	2.6	71	4.3	4.5	4.7	5.4	6.2	6.4	6.6
36	1.2	1.4	1.5	2.0	2.4	2.6	2.7	72	4.4	4.6	4.8	5.5	6.3	6.5	6.7
37	1.3	1.4	1.6	2.1	2.5	2.7	2.8	73	4.5	4.7	4.9	5.6	6.4	6.6	6.8
38	1.4	1.5	1.7	2.2	2.7	2.8	2.9	74	4.6	4.8	5.0	5.7	6.5	6.7	6.9
39	1.5	1.6	1.8	2.3	2.8	2.9	3.0	75	4.7	4.8	5.1	5.8	6.6	6.8	7.0
40	1.6	1.7	1.9	2.4	2.9	3.0	3.1	76	4.7	4.9	5.2	5.9	6.7	6.9	7.1
41	1.7	1.8	1.9	2.5	3.0	3.1	3.2	77	4.8	5.0	5.2	6.0	6.8	7.0	7.2
42	1.8	1.9	2.0	2.6	3.1	3.2	3.4	78	4.9	5.1	5.3	6.1	6.9	7.1	7.3
43	1.8	2.0	2.1	2.7	3.2	3.3	3.5	79	5.0	5.2	5.4	6.2	7.0	7.2	7.4
44	1.9	2.1	2.2	2.8	3.3	3.4	3.6	80	5.1	5.3	5.5	6.3	7.1	7.3	7.5
45	2.0	2.2	2.3	2.9	3.4	3.6	3.7	81	5.2	5.4	5.6	6.4	7.2	7.5	7.7
46	2.1	2.2	2.4	3.0	3.5	3.7	3.8	82	5.3	5.5	5.7	6.5	7.3	7.6	7.8
47	2.2	2.3	2.5	3.1	3.6	3.8	3.9	83	5.4	5.6	5.8	6.6	7.4	7.7	7.9
48	2.3	2.4	2.6	3.2	3.7	3.9	4.0	84	5.4	5.6	5.9	6.7	7.5	7.8	8.0
49	2.4	2.5	2.7	3.3	3.8	4.0	4.1	85	5.5	5.7	6.0	6.8	7.6	7.9	8.1
50	2.5	2.6	2.8	3.3	3.9	4.1	4.2	86	5.6	5.8	6.1	6.9	7.8	8.0	8.2
51	2.5	2.7	2.9	3.4	4.0	4.2	4.3	87	5.7	5.9	6.2	7.0	7.9	8.1	8.3
52	2.6	2.8	3.0	3.5	4.1	4.3	4.5	88	5.8	6.0	6.2	7.1	8.0	8.2	8.4
53	2.7	2.9	3.0	3.6	4.2	4.4	4.6	89	5.9	6.1	6.3	7.2	8.1	8.3	8.5
54	2.8	3.0	3.1	3.7	4.4	4.5	4.7	90	6.0	6.2	6.4	7.3	8.2	8.4	8.6
55	2.9	3.1	3.2	3.8	4.5	4.6	4.8	91	6.1	6.3	6.5	7.4	8.3	8.5	8.8
56	3.0	3.1	3.3	3.9	4.6	4.7	4.9	92	6.1	6.4	6.6	7.5	8.4	8.6	8.9
57	3.1	3.2	3.4	4.0	4.7	4.9	5.0	93	6.2	6.5	6.7	7.6	8.5	8.8	9.0
58	3.2	3.3	3.5	4.1	4.8	5.0	5.1	94	6.3	6.5	6.8	7.7	8.6	8.9	9.1
59	3.3	3.4	3.6	4.2	4.9	5.1	5.2	95	6.4	6.6	6.9	7.8	8.7	9.0	9.2
60	3.3	3.5	3.7	4.3	5.0	5.2	5.3	96	6.5	6.7	7.0	7.9	8.8	9.1	9.3

aortic valve and pulmonary valve were obtained by minutely maneuvering the volume dataset in both the panel A and B of the multiplanar view. The diameters of the aortic valve and pulmonary valve annuli were measured from inner edge to inner edge at end-systole.

Statistical methodologies were utilized to develop Z-scores reference intervals using the statistical package for the social sciences (SPSS) version 17.0 (Chicago, USA) following the instruction published by Royston and Wright (1998). Briefly, models for the dependence of aortic and pulmonary valve diameter on GA and on biparietal diameter (BPD), and their standard deviations, were selected by the successive testing of the highest-order coefficient of cubic, quadratic and linear models for significant difference from zero. Normality and goodness of fitted regression models were assessed by examination of the scatter patterns of points relative to fitted means and standard deviations expressed as Z scores (measured value − estimated mean/estimated SD). Z scores were tested for normality using Sharpiro–Wilk W test and QQ plots. The centile curves and nomograms were constructed from the formula: Centile = mean + $K \times$ SD (K was the corresponding centile of the standard Gaussian distribution).

RESULTS

A total of 654 4D-STIC volume datasets were acquired and available for off-line analysis; however, only 606 volume datasets were satisfactorily measured for the aortic and pulmonary valve diameter. The mean maternal age was 27 ± 6 years (range 15–43) and most of them (59%) were nulliparous. The distribution of the participants in each GA is shown in Table 1.

A best described linear regression model predicted mean values and standard deviations (SD) of both fetal aortic and pulmonary valve diameter based on gestational weeks and BPD (Tables 1–3 and Figure 2). The best fitted equations for SD were derived from regression of scaled absolute residuals (SARs) which were calculated as: SAR = 1.25 × Abs (measured value − predicted value). These were found to be suitably represented by a linear relationship with GA as well as BPD, resulting in the equation for the SD as presented in

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Table 3—Pulmonary valve diameter (mm) as a function of biparietal diameter (mm)

BPD	2.5th	5th	10th	50th	90th	95th	97.5th	BPD	2.5th	5th	10th	50th	90th	95th	97.5th
25	0.3	0.5	0.6	1.0	1.5	1.6	1.7	61	3.8	4.0	4.2	4.9	5.6	5.8	6.0
26	0.4	0.6	0.7	1.1	1.6	1.7	1.8	62	3.9	4.1	4.3	5.0	5.8	6.0	6.1
27	0.5	0.7	0.8	1.2	1.7	1.8	1.9	63	4.0	4.2	4.4	5.1	5.9	6.1	6.3
28	0.6	0.8	0.9	1.3	1.8	1.9	2.1	64	4.1	4.3	4.5	5.2	6.0	6.2	6.4
29	0.7	0.9	1.0	1.5	1.9	2.1	2.2	65	4.2	4.4	4.6	5.4	6.1	6.3	6.5
30	0.8	1.0	1.1	1.6	2.0	2.2	2.3	66	4.3	4.5	4.7	5.5	6.2	6.4	6.6
31	0.9	1.0	1.2	1.7	2.2	2.3	2.4	67	4.4	4.6	4.8	5.6	6.3	6.5	6.7
32	1.0	1.1	1.3	1.8	2.3	2.4	2.5	68	4.5	4.7	4.9	5.7	6.4	6.7	6.9
33	1.1	1.2	1.4	1.9	2.4	2.5	2.7	69	4.6	4.8	5.0	5.8	6.6	6.8	7.0
34	1.2	1.3	1.5	2.0	2.5	2.7	2.8	70	4.7	4.9	5.1	5.9	6.7	6.9	7.1
35	1.3	1.4	1.6	2.1	2.6	2.8	2.9	71	4.8	5.0	5.2	6.0	6.8	7.0	7.2
36	1.4	1.5	1.7	2.2	2.7	2.9	3.0	72	4.9	5.1	5.3	6.1	6.9	7.1	7.3
37	1.5	1.6	1.8	2.3	2.9	3.0	3.1	73	5.0	5.2	5.4	6.2	7.0	7.3	7.5
38	1.6	1.7	1.9	2.4	3.0	3.1	3.3	74	5.1	5.3	5.5	6.3	7.1	7.4	7.6
39	1.7	1.8	2.0	2.5	3.1	3.2	3.4	75	5.2	5.4	5.6	6.4	7.3	7.5	7.7
40	1.8	1.9	2.1	2.6	3.2	3.4	3.5	76	5.3	5.5	5.7	6.5	7.4	7.6	7.8
41	1.9	2.0	2.2	2.8	3.3	3.5	3.6	77	5.4	5.6	5.8	6.7	7.5	7.7	7.9
42	2.0	2.1	2.3	2.9	3.4	3.6	3.7	78	5.5	5.7	5.9	6.8	7.6	7.8	8.1
43	2.1	2.2	2.4	3.0	3.6	3.7	3.9	79	5.6	5.8	6.0	6.9	7.7	8.0	8.2
44	2.2	2.3	2.5	3.1	3.7	3.8	4.0	80	5.7	5.9	6.1	7.0	7.8	8.1	8.3
45	2.3	2.4	2.6	3.2	3.8	4.0	4.1	81	5.8	6.0	6.2	7.1	8.0	8.2	8.4
46	2.4	2.5	2.7	3.3	3.9	4.1	4.2	82	5.9	6.1	6.3	7.2	8.1	8.3	8.5
47	2.5	2.6	2.8	3.4	4.0	4.2	4.3	83	6.0	6.2	6.4	7.3	8.2	8.4	8.7
48	2.6	2.7	2.9	3.5	4.1	4.3	4.5	84	6.1	6.3	6.5	7.4	8.3	8.6	8.8
49	2.7	2.8	3.0	3.6	4.2	4.4	4.6	85	6.2	6.4	6.6	7.5	8.4	8.7	8.9
50	2.8	2.9	3.1	3.7	4.4	4.5	4.7	86	6.3	6.5	6.7	7.6	8.5	8.8	9.0
51	2.9	3.0	3.2	3.8	4.5	4.7	4.8	87	6.3	6.6	6.8	7.7	8.6	8.9	9.1
52	3.0	3.1	3.3	3.9	4.6	4.8	4.9	88	6.4	6.7	6.9	7.8	8.8	9.0	9.3
53	3.1	3.2	3.4	4.1	4.7	4.9	5.1	89	6.5	6.8	7.0	8.0	8.9	9.1	9.4
54	3.2	3.3	3.5	4.2	4.8	5.0	5.2	90	6.6	6.9	7.1	8.1	9.0	9.3	9.5
55	3.3	3.4	3.6	4.3	4.9	5.1	5.3	91	6.7	7.0	7.2	8.2	9.1	9.4	9.6
56	3.3	3.5	3.7	4.4	5.1	5.3	5.4	92	6.8	7.1	7.3	8.3	9.2	9.5	9.7
57	3.4	3.6	3.8	4.5	5.2	5.4	5.5	93	6.9	7.2	7.4	8.4	9.3	9.6	9.9
58	3.5	3.7	3.9	4.6	5.3	5.5	5.7	94	7.0	7.3	7.5	8.5	9.5	9.7	10.0
59	3.6	3.8	4.0	4.7	5.4	5.6	5.8	95	7.1	7.4	7.6	8.6	9.6	9.9	10.1
60	3.7	3.9	4.1	4.8	5.5	5.7	5.9	96	7.2	7.5	7.7	8.7	9.7	10.0	10.2

Table 4. These equations were validated by constructing a Z score for each parameter. The normalcy of the Z scores was evident in a QQ plot. The Sharpiro–Wilk tests showed normality in distribution. Further, the Z scores were evenly distributed above and below zero across the entire range of GAs and BPDs. Finally, Z scores which were outside of the range ± 1.645 did not significantly differ from the expected 10% of the values ($P = 0.46$).

Z scores for each actual measured value could be calculated using the reference equation as follows: Z score = (measured value − predicted value)/predicted SD

For example, measured BPD = 30 mm and aortic valve diameter = 3.2 mm

Using formula in Table 4: Predicted aortic valve = $-1.5952 + 0.0989(30) = 1.3718$; Predicted SD of aortic valve = $0.1672 + 0.00572(30) = 0.3388$; Z score = $(3.2 - 1.3718)/0.3388 = +5.396$.

Therefore, a fetus with aortic valve at +5.396 SD above the predicted mean aortic diameter for a BPD of 30 mm would be considered to have enlarged valves.

Centile models of aortic and pulmonary valve diameter as a function of GA in weeks and BPD in millimeter are shown in Tables 2–4.

DISCUSSION

According to the International Society of Ultrasound in Obstetrics and Gynecology guideline for performing fetal heart examination, views of the left and right outflow tracts are part of the extended basic cardiac examination, which enhances the detection rate for congenital heart anomalies (Anon, 2006). The normal crisscross phenomenon of the great arteries at the ventricular outlets and the equally measured size of aortic and pulmonary valve annulus are essential for minimum criteria of cardiac assessment. An abnormal difference in dimensions of both great arteries would raise suspicion for aortic stenosis, coarctation of aorta, pulmonary atresia or tetralogy of Fallot. Therefore, the accurate measurement of both aorta and pulmonary artery is necessary for the correct diagnosis, and subsequent monitoring. Compared with previous publications on fetal aortic and pulmonary valves normality values derived from 2D fetal echocardiography (DeVore *et al*., 1985; Sharland and Allan, 1992; Shapiro *et al*., 1998; DeVore, 2005; Schneider *et al*., 2005; Lee *et al*., 2010), the values of reference ranges in our study are slightly lower (Table 5). This

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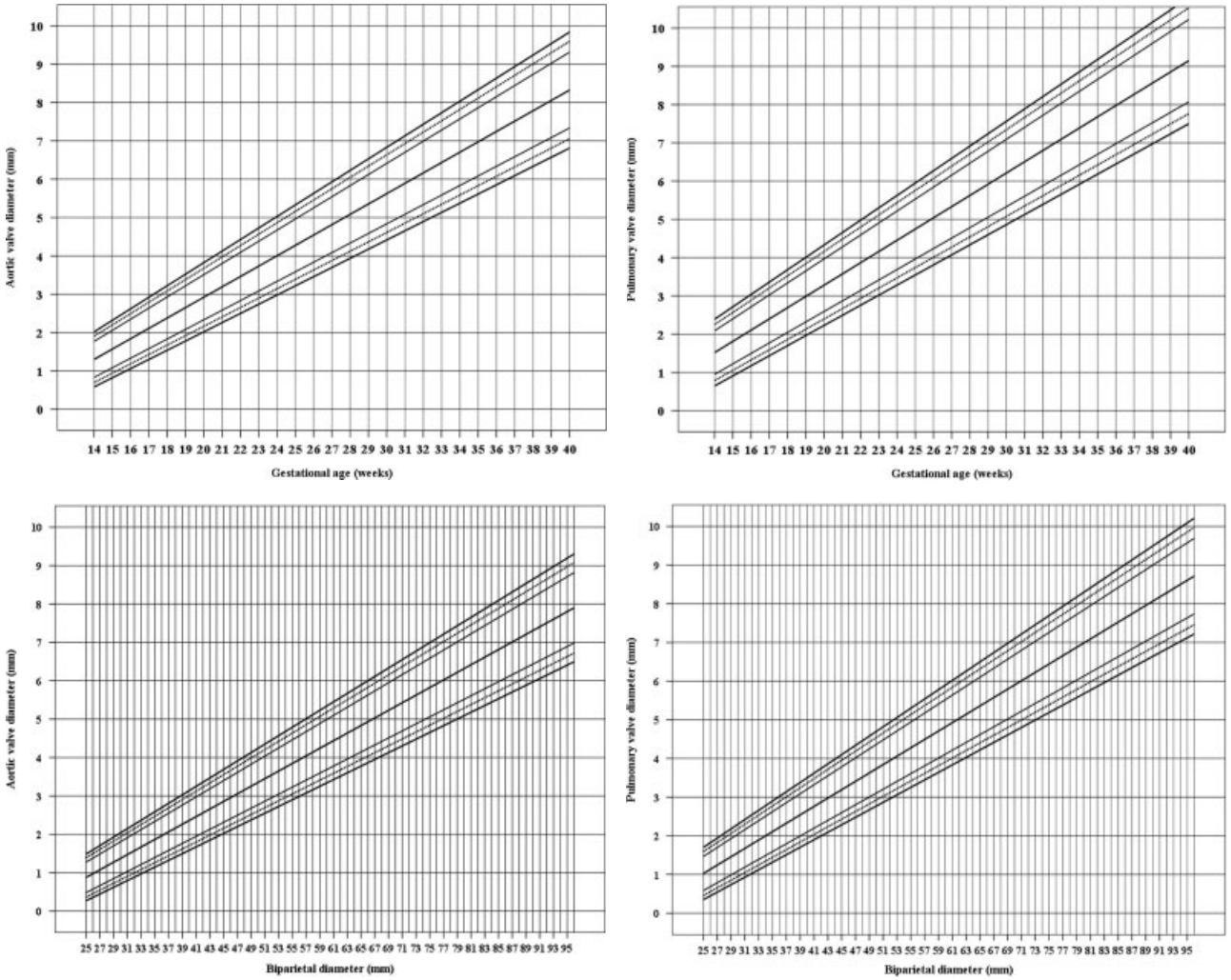

Figure 2—Linear relationship between gestational age in weeks and aortic valve diameter (a) and pulmonary valve diameter (b); between biparietal diameter in millimeter and aortic valve diameter (c) and pulmonary valve diameter (d)

Table 4—Simple linear regression models for prediction mean and SD of fetal aortic and pulmonary valve diameter based on gestational age (GA) and biparietal diameter (BPD)

Parameter	Model derived from regression analysis	Correlation coefficient
Gestational age (GA)		
Aortic valve diameter	$-2.4838 + 0.2702$(GA in weeks)	0.963
SD of aortic valve diameter	$0.1482 + 0.0156$ (GA in weeks)	0.262
Pulmonary valve diameter	$-2.5924 + 0.2935$(GA in weeks)	0.961
SD of pulmonary valve diameter	$-0.2317 + 0.01524$(GA in weeks)	0.242
Biparietal diameter (BPD)	$-1.5952 + 0.0989$ (BPD in mm)	0.970
Aortic valve diameter	$0.1672 + 0.00572$ (BPD in mm)	0.293
SD of aortic valve diameter	$-1.6830 + 0.1083$ (BPD in mm)	0.970
Pulmonary valve diameter	$-0.1971 + 0.0059$ (BPD in mm)	0.277
SD of pulmonary valve diameter		

may be due to racial factors or differences in the techniques used in the measurements.

Unlike most previous studies on reference ranges of cardiac dimensions, in which modeling of the variability was not often considered (even though in the field of fetal size SD almost always changes with GA) (Altman, 1993), our study performed residual analysis to check the proper modeling for the construction of the reference range and emphasized the use of specific statistical methodologies to optimize regression models in predicting the robust Z-scores of the aortic and pulmonary valves. This is because the development of reliable Z-score reference ranges depends on careful examination of the data to verify that residual variation conforms to a

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Table 5—A comparison of aortic valve and pulmonary valve diameters (at 1 and 2 SD) based on gestational age (GA) and biparietal diameter (BPD) among our study (study1), that by Lee *et al*. (study 2), and Schneider *et al*. (study 3)

	Aortic valve						Pulmonary valve					
	Diameter at Z score = 1			Diameter at Z score = 2			Diameter at Z score = 1			Diameter at Z score = 2		
	Study 1	Study 2	Study 3	Study 1	Study 2	Study 3	Study 1	Study 2	Study 3	Study 1	Study 2	Study 3
GA (weeks)												
14	1.67	1.74	2.11	2.03	2.03	2.39	1.96	1.94	2.40	2.41	2.25	2.71
15	1.95	2.04	2.30	2.33	2.35	2.61	2.27	2.28	2.64	2.73	2.61	2.98
16	2.24	2.35	2.49	2.64	2.67	2.83	2.58	2.62	2.88	3.05	2.98	3.25
17	2.52	2.65	2.69	2.94	3.00	3.06	2.89	2.96	3.13	3.38	3.34	3.53
18	2.81	2.96	2.89	3.24	3.32	3.29	3.20	3.29	3.38	3.70	3.70	3.81
19	3.09	3.26	3.10	3.54	3.64	3.52	3.51	3.63	3.63	4.03	4.06	4.10
20	3.38	3.56	3.30	3.84	3.96	3.76	3.81	3.97	3.89	4.35	4.43	4.39
21	3.67	3.87	3.51	4.14	4.28	4.00	4.12	4.31	4.16	4.67	4.79	4.69
22	3.95	4.17	3.73	4.44	4.60	4.24	4.43	4.64	4.43	5.00	5.15	5.00
23	4.24	4.48	3.94	4.74	4.92	4.48	4.74	4.98	4.70	5.32	5.51	5.31
24	4.52	4.78	4.16	5.05	5.24	4.73	5.05	5.32	4.98	5.65	5.88	5.62
25	4.81	5.09	4.38	5.35	5.56	4.98	5.36	5.65	5.27	5.97	6.24	5.94
26	5.10	5.39	4.60	5.65	5.88	5.23	5.67	5.99	5.55	6.29	6.60	6.27
27	5.38	5.70	4.83	5.95	6.21	5.49	5.98	6.33	5.84	6.62	6.96	6.59
28	5.67	6.00	5.05	6.25	6.53	5.75	6.28	6.67	6.14	6.94	7.33	6.93
29	5.95	6.30	5.28	6.55	6.85	6.01	6.59	7.00	6.44	7.27	7.69	7.26
30	6.24	6.61	5.52	6.85	7.17	6.27	6.90	7.34	6.74	7.59	8.05	7.60
31	6.52	6.91	5.75	7.16	7.49	6.53	7.21	7.68	7.04	7.91	8.41	7.95
32	6.81	7.22	5.98	7.46	7.81	6.80	7.52	8.02	7.35	8.24	8.78	8.30
33	7.10	7.52	6.22	7.76	8.13	7.07	7.83	8.35	7.66	8.56	9.14	8.65
34	7.38	7.83	6.46	8.06	8.45	7.34	8.14	8.69	7.98	8.89	9.50	9.01
35	7.67	8.13	6.70	8.36	8.77	7.62	8.45	9.03	8.30	9.21	9.87	9.37
36	7.95	8.44	6.94	8.66	9.09	7.89	8.75	9.36	8.62	9.53	10.23	9.73
37	8.24	8.74	7.19	8.96	9.42	8.17	9.06	9.70	8.95	9.86	10.59	10.10
38	8.52	9.05	7.43	9.27	9.74	8.45	9.37	10.04	9.28	10.18	10.95	10.47
39	8.81	9.35	7.68	9.57	10.06	8.73	9.68	10.38	9.61	10.51	11.32	10.84
40	9.10	9.65	7.93	9.87	10.38	9.02	9.99	10.71	9.94	10.83	11.68	11.22
BPD (mm)												
20	0.66	0.73	1.48	0.95	0.96	1.93	0.80	1.97	0.82	1.11	1.03	2.21
25	1.19	1.25	1.87	1.50	1.52	2.43	1.37	2.53	1.40	1.71	1.66	2.85
30	1.71	1.78	2.26	2.05	2.08	2.94	1.94	3.11	1.99	2.31	2.29	3.49
35	2.23	2.31	2.66	2.60	2.64	3.45	2.51	3.70	2.57	2.91	2.92	4.16
40	2.76	2.83	3.05	3.15	3.20	3.96	3.08	4.30	3.16	3.52	3.56	4.83
45	3.28	3.36	3.45	3.70	3.75	4.48	3.65	4.91	3.74	4.12	4.19	5.52
50	3.80	3.89	3.85	4.26	4.31	5.00	4.22	5.52	4.33	4.72	4.82	6.21
55	4.33	4.41	4.25	4.81	4.87	5.52	4.80	6.15	4.91	5.32	5.45	6.91
60	4.85	4.94	4.65	5.36	5.43	6.04	5.37	6.78	5.50	5.92	6.08	7.62
65	5.37	5.47	5.05	5.91	5.99	6.56	5.94	7.42	6.09	6.52	6.71	8.34
70	5.90	5.99	5.46	6.46	6.54	7.09	6.51	8.07	6.67	7.12	7.34	9.07
75	6.42	6.52	5.86	7.01	7.10	7.61	7.08	8.72	7.26	7.72	7.97	9.80
80	6.94	7.05	6.27	7.57	7.66	8.14	7.65	9.38	7.84	8.32	8.60	10.54
85	7.46	7.57	6.68	8.12	8.22	8.67	8.22	10.04	8.43	8.92	9.23	11.29
90	7.99	8.10	7.09	8.67	8.78	9.20	8.79	10.71	9.01	9.52	9.87	12.04
95	8.51	8.62	7.49	9.22	9.33	9.73	9.36	11.38	9.60	10.12	10.50	12.79

normal distribution. The residuals (differences between the predicted and observed values) were analyzed to calculate SD and then tested to determine whether their residuals conformed to a normal distribution. Residual analysis must be evaluated across the range of GA or BPD to determine whether and how SD varies, otherwise the denominator used in the calculation of a Z-score may be unreliable.

Additionally, to avoid improper plane and cardiac phase in measurement secondary to difficulties with 2D ultrasound in some cases, we used 4D-ultrasound with STIC to control all axes of the virtual cardiac dimension and cycles. With STIC, the acquired volume dataset allows image collection in all planes including the entire cardiac cycle information which can be displayed and rearranged after the patient scan. The accuracy of the measurement is theoretically better than that derived from 2D ultrasound since we could rotate the volume dataset and move the reference dot in three axes to control the display of the aorta and pulmonary artery. The off-line analysis permits the examiner to navigate thoroughly through the heart in

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all dimensions and in every phases of the cardiac cycle; thus the precise areas for the measurement are certainly defined leading to the accurate measurement (Chaoui and Heling, 2005; Goncalves *et al*., 2006). In this study, the left and right ventricular outflow tracts were perfectly adjusted via multiplanar display and the exact end-systolic cardiac phase was correctly selected. Visualization of the three orthogonal planes at the same time can reassure regarding the correct position for measurement, the greatest dimensions of the valves at the correct cardiac phase. Additionally, high reliability may be associated with off-line examination without time limits allowing to obtain the best image. These reference ranges may probably be more appropriate for evaluation and facilitate the diagnosis in earlier gestation.

The strength of this study included a large sample size, the measurements based on innovative STIC, and providing Z scores derived from residual analysis with checking models. Despite a number of normal fetal cardiac dimension reports, several reference ranges do not provide Z-score assessment. The qualitative assessment using centiles has been demonstrated to be less accurate than the quantitative assessment using Z-scores. The principal concepts are the appropriate adjustment of both mean and standard deviation curves (Royston and Wright, 1998). This study provides predicted mean values and SD for each GA and BPD range, permitting the Z scores calculation. These reference ranges may be especially helpful for detection and monitoring of suspected abnormalities of the fetal heart especially the outflow tracts.

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ORIGINAL ARTICLE

Fetal liver length measurement at mid-pregnancy among fetuses at risk as a predictor of hemoglobin Bart's disease

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Objective: To assess the effectiveness of liver length measurement in predicting hemoglobin (Hb) Bart's disease among fetuses at risk at mid-pregnancy.

Study Design: Pregnant women at risk of fetal Hb Bart's disease at 18 to 22 weeks of gestation were enrolled in the study. All of them underwent liver length measurement just before cordocentesis for fetal blood analysis. The final diagnosis used as a gold standard was based on fetal Hb typing using high-performance liquid chromatography.

Result: A total of 334 pregnant women were recruited into the study. The prevalence of fetal Hb Bart's disease was 26.1% (87 of 334 fetuses). The sensitivity, specificity, as well as positive and negative predictive values of liver length in the prediction of affected fetuses were 71.3, 95.5, 84.9 and 90.4%, respectively. Overall, 24 fetuses (27.6%) had some degree of early hydrops fetalis. Of these, 20 had hepatomegaly as well.

Conclusion: Liver length measurements at mid-pregnancy may be helpful in predicting affected fetuses among pregnancies at risk. Normal liver length measurement is associated with a very low risk of the disease. This information may help couples decide on whether to opt for either invasive diagnosis or a noninvasive approach, especially when used as an adjunct to other tests.

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Keywords: Hb Bart's disease; liver length; prenatal diagnosis; ultrasound

Introduction

Hemoglobin (Hb) Bart's disease (homozygous α-thalassemia-1) is the most common cause of hydrops fetalis in Southeast Asia.[1–3] At present, this disease is more prevalent in other parts of the world because of population migrations. Fetuses with this disorder are uniformly lethal. Nevertheless, prenatal diagnosis is imperative because serious maternal complications are often encountered in pregnant women with Hb Bart's hydrops fetalis, including severe preeclampsia, dystocia, postpartum hemorrhage due to placentomegaly and psychological burden for carrying a nonsurviving fetus to term. Therefore, early diagnosis and early termination of the pregnancy before development of hydrops is essential. At present, although several sonographic markers have been proven to be useful in predicting fetal Hb Bart's disease before hydropic changes occur,[4,5] the efficacy of liver measurement for this purpose has never been thoroughly evaluated. Surprisingly, although it has long been known that hepatomegaly associated with extramedullary erythropoiesis is always seen in hydropic fetuses due to Hb Bart's disease, it has never been tested whether liver length measurement is helpful in early prenatal detection of this disorder, in spite of its simplicity to measure. Theoretically, liver length measurement is particularly helpful in the prediction of severe anemia occurring in early gestation such as Hb Bart's disease as fetal anemia can develop in as early as 12 to 13 weeks of gestation.[6,7] Roberts *et al*.[8] evaluated fetal liver length on 53 examinations conducted in 21 isoimmunized pregnancies and found that all fetuses with a Hb of <10.0 g per 100 ml had a liver length that was greater than the ninetieth percentile, suggesting that liver length is a useful indicator of the degree of fetal anemia in isoimmunized pregnancies. This is consistent with a preliminary report by Vintzileos *et al*.[9] Similarly, fetuses with Hb Bart's disease, which is associated with severe anemia from early gestation, seem to have hepatomegaly at the same time. Although the classic hydropic changes due to Hb Bart's disease are likely to occur after 20 weeks,[10] our experience of ultrasound examinations at 18 to 22 weeks suggests that fetal liver length measurement may be useful in distinguishing normal from affected pregnancies. We hypothesize that liver length measurement may be useful in identifying fetal Hb Bart's disease among fetuses at risk before the development of frank hydrops fetalis. The objective of this study was to evaluate the efficacy of liver length at mid-pregnancy in predicting fetal Hb Bart's disease.

Methods

This prospective diagnostic study was undertaken at the Maharaj Nakorn Chiang Mai Hospital (Department of Obstetrics and

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Gynecology, Chiang Mai University, Thailand), with approval of the research ethical committee. Pregnant women were recruited into the study with written informed consent. The inclusion criteria included the following: (1) singleton pregnancies at risk of having fetuses with Hb Bart's disease (both of the couples were carriers of α-thalassemia-1, based on PCR for Southeast Asian type); (2) gestational age of 18 to 22 weeks, based on a reliable last menstrual period or fetal sonographic biometry in the first half of pregnancy; and (3) known definite diagnosis of Hb Bart's disease or non-Hb Bart's disease, based on cordocentesis and fetal blood analysis with high-performance liquid chromatography. The exclusion criteria were as follows: (1) fetal anomalies or chromosomal abnormalities and (2) unknown final fetal diagnosis in terms of the presence or absence of Hb Bart's disease. All ultrasound examinations were performed just before cordocentesis by the authors, using a real-time scanner with Aloka SSD alpha-10 machine (Aloka Co, Tokyo, Japan) or GE Voluson E8 (GE Healthcare, Wauwatosa, WI, USA), equipped with a trans-abdominal curvilinear transducer of frequency 2 to 5 MHz. On ultrasound examination, liver length measurement, standard fetal biometry and anomaly screening were performed. The steps in liver length measurement were as follows:[8,11] (1) The fetal upper abdomen at the level of the stomach was first demonstrated on an axial view and the liver was visualized as the most visible part of the abdomen. (2) To appropriately measure the right lobe of the liver, fine adjustment and rotating the transducer was performed to obtain the proper plane for measurement, and the aorta was first imaged in the longitudinal plane. The transducer is then moved parallel to this plane until the tip of the right lobe and the right hemidiaphragm were imaged. Occasionally, the transducer had to be tipped to visualize both the diaphragm and the right lobe of the liver. This was a coronal image of the abdomen. (3) Fetal liver length was measured from the region where the right margin of the heart was in contact with the hemidiaphragm (top of the right hemidiaphragm) to the tip of the right lobe. Liver length was measured by placing electronic calipers as shown in Figure 1. The measurement usually took <5 min; 2 to 3 min in most cases. The three best measurements were digitally recorded in the computerized record form and subsequently averaged for analysis. In addition, middle cerebral artery peak systolic velocity (MCA-PSV) was also measured using a standard technique described elsewhere.[12] All cordocenteses were performed immediately after ultrasound examination. Baseline demographic and obstetric data of pregnant women were also recorded on the patient's digital record forms at the same time. The pregnant women were treated as standard antenatal care. Hepatomegaly or abnormally enlarged liver size was defined by a liver length larger than the cutoff value based on the receiver–operator characteristic curve. The stored data were subsequently analyzed for the efficacy of fetal liver length in predicting fetal Hb Bart's disease, using the statistical package for the social sciences (SPSS) version 17.0 (SPSS, Chicago, IL, USA).

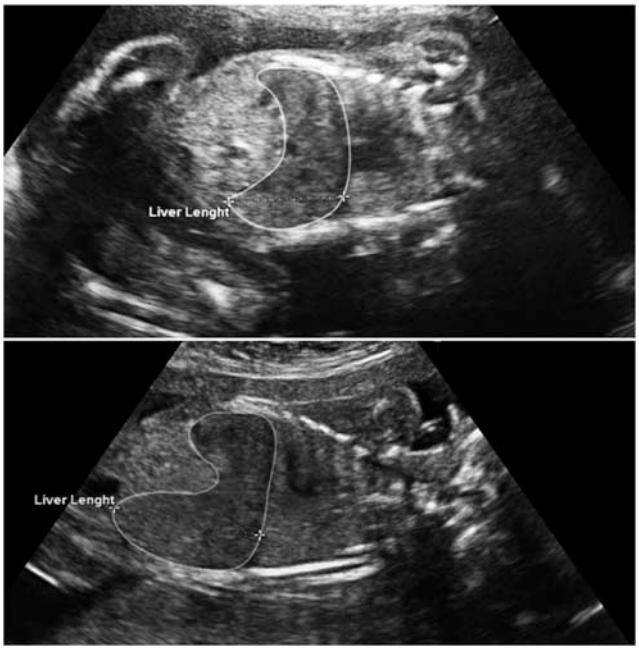

Figure 1 Measurement of the fetal liver length of a normal fetus (upper) and a fetus with hemoglobin Bart's disease (lower) at 21 weeks of gestation.

For sample size estimation, to gain a confidence level of 95% for a detection rate of at least 60% and a maximum allowable error (precision) rate of 10%, the study should include affected fetuses of at least 48 cases. Measurement outcomes were presented as sensitivity, specificity, as well as positive and negative predictive values.

Results

Over the study period (June 2007 to February 2010), 355 pregnant women at risk for having fetuses with Hb Bart's disease were recruited into the study. Overall, 334 of them were sonographically measured for liver length at 18 to 22 weeks and underwent cordocentesis with the available final diagnosis. Of 334 fetuses, 87 were finally proven to be affected by Hb Bart's disease, and the remaining fetuses were unaffected. The mean maternal age ($\pm$ s.d.) was 27.02 $\pm$ 6.7 years (range: 13 to 48). The mean gestational age ($\pm$ s.d.) was 19.87 $\pm$ 1.3 weeks (range: 18 to 22 weeks). Of them, 42.5% were nulliparous and 57.5% were multiparous. No serious maternal complications, such as pregnancy-induced hypertension, were observed at mid-pregnancy.

The receiver–operator characteristic curve of liver length in predicting Hb Bart's disease was analyzed as shown in Figure 2. The area under curve was 0.922 (95% confidence interval: 0.890 to 0.954) and the best cutoff was at a liver length of 27.0 mm. The diagnostic indices of liver length measurement in differentiating fetuses with Hb Bart's disease from unaffected fetuses are summarized in Table 1. The liver length had a sensitivity of 71.3%

and a specificity of 95.5%. Overall, 24 fetuses (27.6%) had some degree of hydrops fetalis, such as minimal ascites or pleural effusion. Notably, of these, 20 showed hepatomegaly as well. When fetuses with hydrops fetalis were excluded, sensitivity decreased to 66.7%, whereas specificity remained the same, as presented in Table 1. MCA-PSV, defined as abnormal if >1.5 MoM, had a sensitivity of 91.0% and a specificity of 98.2% as shown in Table 1. When combined, liver length and MCA-PSV, considered abnormal if either of them was abnormal, the combination had a sensitivity of 96.2% and a specificity of 94.1%. When analyzed separately for a specific gestational week, the accuracy of liver length in prediction was not significantly different as shown in Table 2.

Discussion

The fetal liver is a major site of hematopoiesis in the second trimester and is a primary site of extramedullary hematopoiesis in fetuses complicated by anemia, in particular Hb Bart's disease or Rh isoimmunization, leading to enlargement of the liver.[8]

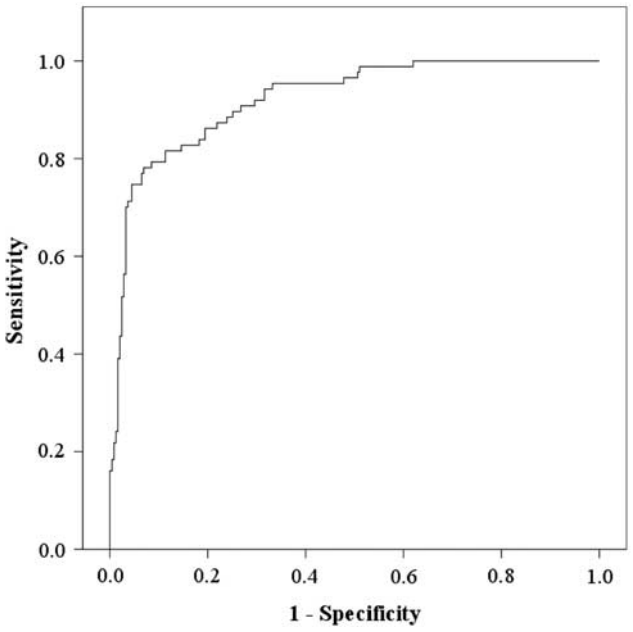

Figure 2 ROC curve of liver length in predicting Hb Bart's disease; area under curve = 0.922 (95% confidence interval: 0.890 to 0.954), the best cutoff is at a liver length of 27.0 mm. Hb, hemoglobin; ROC, receiver–operator characteristic.

Therefore, measurement of fetal liver length can theoretically be useful in the diagnosis of fetal anemia owing to various causes. In spite of being known for a long time that hepatomegaly is closely associated with the degree of fetal anemia, no study on liver size in predicting fetal Hb Bart's disease has been reported. To our best knowledge, this is the first study in the evaluation of this simple and practical technique in differentiating affected from unaffected fetuses.

On the basis of a comparison of fetal liver length of 21 isoimmunized pregnancies and 350 measurements in normal pregnancies, Roberts *et al*.[8] found a good correlation between liver length and fetal Hb level ($r = 0.794$, $P<0.001$) and between liver length and reticulocyte count ($r = 0.721$, $P<0.001$). All fetuses with Hb <10.0 g per 100 ml had a liver length greater than the ninetieth percentile. They concluded that liver length measurement seems to be a useful indicator of the degree of fetal anemia in isoimmunized pregnancies. However, although our findings were consistent with those reported by Roberts *et al.*, liver length measurement in our study was not as highly effective as seen in their report. This may be because of the fact that liver length at 18 to 22 weeks of gestation may be not as sensitive in differentiating affected from unaffected fetuses as in later pregnancy like in the report by Roberts *et al*. At mid-pregnancy, most fetuses with Hb Bart's disease do not develop hydropic changes, and fetal anemia may not be so severe at this stage of pregnancy. We believe that in advanced gestational age when fetal anemia becomes more severe, liver length is likely to have higher sensitivity and specificity in predicting anemia.

Table 2 Diagnostic indices of liver length in predicting Hb Bart's disease at various gestational weeks, based on the cutoff at 27.0 mm

GA week	N	*Sensitivity*[a]	*Specificity*[a]	*PPV*[a]	*NPV*[a]
18	70	73.3% (11/15)	94.5% (52/55)	78.6% (11/14)	92.9% (56/70)
19	66	69.2% (9/13)	96.2% (51/53)	81.8% (9/11)	92.7% (51/55)
20	82	71.4% (20/28)	94.4% (51/54)	87.0% (20/23)	86.4% (51/59)
21	72	70.0% (7/10)	96.8% (60/62)	77.8% (7/9)	95.2% (60/63)
22	44	71.4% (15/21)	95.7% (22/23)	93.8% (15/16)	78.6% (22/28)

Abbreviations: GA, gestational age; Hb, hemoglobin; NPV, negative predictive value; PPV, positive predictive value.
[a]No statistical difference between gestational age group; χ^2, $P>0.05$.

Table 1 Diagnostic indices of liver length and middle cerebral peak systolic velocity (MCA-PSV) in predicting Hb Bart's disease

	Test	N	*Sens*	*Spec*	*PPV*	*NPV*
(1)	Liver length (total fetuses)	334	71.3% (62/87)	95.5% (236/247)	84.9% (62/73)	90.4% (236/261)
(2)	Liver length (excluded fetal hydrops)	310	66.7% (42/63)	95.5% (236/247)	79.2% (42/53)	91.8% (236/257)
(3)	MCA-PSV (total fetuses)	298	91.0% (71/78)	98.2% (216/220)	94.7% (71/75)	96.9% (216/223)
(4)	MCA-PSV and/or liver length (total fetuses)	298	96.2% (75/78)	94.1% (207/220)	85.2% (75/88)	98.6% (207/210)

Abbreviations: Hb, hemoglobin; NPV, negative predictive value; PPV, positive predictive value; Sens, sensitivity; Spec, specificity.

Among pregnancies at risk, at mid-pregnancy, liver length can predict fetuses with Hb Bart's disease with a sensitivity of ~70% and a specificity of 95%. A relatively high specificity indicates that normal liver length at 18 to 22 weeks reduces the probability of being affected by the disease and may obviate the need for invasive procedures to confirm normality, especially in case of no other sonomarkers such as cardiomegaly or placentomegaly, although follow-up sonographic examinations are still required. A sensitivity of 70% suggests that liver length measurement may not be a good screening tool in identifying affected fetuses when compared with MCA-PSV as indicated in this study, because 30% of the affected fetuses will be missed. However, this may be used as an adjunct to other ultrasound markers to gain confidence in predicting fetal anemia, especially in fetuses at risk. When combined with MCA-PSV, liver length may increase sensitivity, although it may slightly compromise specificity. Therefore, measurement of liver length should not be used as a primary tool in the diagnosis of fetal Hb Bart's disease, as several affected fetuses can be missed during diagnosis. Nevertheless, the measurement is simple and easy to be trained, and unlike MCA-PSV, it still seems to be helpful in general practice, especially in the area of high prevalence. This study demonstrated that liver length at different gestational ages during 18 to 22 weeks yields no significant difference in prediction. Therefore, for convenience in clinical use, the same cutoff (27 mm) may be used.

The strengths of this study include (1) a large sample size of pregnant women at risk and (2) the fact that the examiners were blind to the final diagnosis at the time of ultrasound examination, as the measurements were performed just before cordocentesis. Nevertheless, bias might have existed in the measurement of liver length. The measurement was not a completely blind method as the examiner could appreciate the morphology of the fetuses, especially cardiomegaly on sonographic imaging. Therefore, early hydropic signs in affected fetuses, such as cardiomegaly, could have been visible in some cases and the diagnosis of Hb Bart's disease in such fetuses might have been anticipated. Some other limitations should be mentioned, including that interobserver variation among the examiners was not tested. In addition, the measurement may sometimes be difficult in some cases in which the lower border of the liver was unclear and was not always easily discriminated from the surrounding structures, especially using ultrasound machines of earlier models.

In conclusion, liver length assessment at mid-pregnancy can be helpful in predicting fetuses affected by Hb Bart's disease, although the accuracy may be not excellent. Among couples at risk with normal liver size, the risk of having an affected child is much lower, whereas hepatomegaly places the fetuses to be at a higher risk. This information may help couples decide on whether to opt for further invasive diagnosis or a noninvasive approach, especially when used as an adjunct to other tests.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

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Fetal Splenic Artery Peak Velocity (SPA-PSV) at Mid-Pregnancy as a Predictor of Hb Bart's Disease

Spitzengeschwindigkeit in der fetalen A. lienalis (SPA-PSV) in der Schwangerschaftsmitte als Prädiktor der Hb-Bart-Erkrankung

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Key words
- pregnancy
- fetus
- ultrasound Doppler

Zusammenfassung

Ziel: Bewertung der Treffsicherheit der A.-lienalis-Maximalgeschwindigkeit zur Prädiktion einer fetalen Hämoglobin(Hb-)Bart-Erkrankung bei gefährdeten Feten in der Mitte der Schwangerschaft.

Material und Methoden: Schwangere Frauen mit dem Risiko eines Hb-Bart-erkrankten Fetus wurden in der 18.–22. Schwangerschaftswoche (SSW) in die Studie aufgenommen und erhielten vor Chordozentese eine Messung der SPA-PSV. Die definitive Diagnose, die als Goldstandard herangezogen wurde, basierte auf fetaler Hb-Typisierung mittels Hochleistungsflüssigkeitschromatografie (HPLC).

Ergebnisse: Insgesamt wurden 136 Feten aus 132 Einlingsschwangerschaften und 2 Zwillingsschwangerschaften in die Studie eingeschlossen. Das mittlere maternale Alter war 28,7 ± 5,4 Jahre, das mittlere Gestationsalter war 19,1 ± 1,02 Wochen, und die Inzidenz einer Hb-Bart-Erkrankung lag bei 23,5% (32 Feten). Unter Verwendung des 1,51-fachem Medianwerts (MoM) der SPA-PSV als Diskriminationswert lag die Sensitivität, Spezifizität, der positiv prädiktive Wert und der negativ prädiktive Wert der SPA-PSV zur Identifikation von betroffenen Feten bei 84,4% (32 von 36 Fällen), 98,1%, 93,1% bzw. 95,3%. Fast alle gesunden Feten hatten eine normale SPA-PSV.

Schlussfolgerung: SPA-PSV-Messungen in der Mitte der Schwangerschaft können als ergänzende Methode zur Identifizierung von Feten mit Hb-Bart-Erkrankung herangezogen werden und haben eine hohe, wenngleich nicht perfekte Treffsicherheit. Die Methode könnten helfen, bei manchen Feten das Risiko nicht erforderlicher Chordozentesen zu reduzieren.

Abstract

Purpose: To determine the accuracy of splenic artery peak systolic velocity (SPA-PSV) in predicting fetal hemoglobin (Hb) Bart's disease at mid-pregnancy among fetuses at risk.

Materials and Methods: Pregnant women at risk of having a fetus with Hb Bart's disease were recruited into the study at 18 – 22 weeks of gestation and underwent SPA-PSV measurement before cordocentesis. The final diagnosis used as a gold standard was based on fetal hemoglobin typing using high performance liquid chromatography (HPLC).

Results: A total of 136 fetuses from 132 singleton pregnancies and 2 twin pregnancies were recruited into the study. The mean maternal age was 28.7 ± 5.4 years, the mean gestational age was 19.1 ± 1.02 weeks, and the incidence of Hb Bart's disease was 23.5% (32 fetuses). Using SPA-PSV above 1.51 Multiple of Median (MoM) as a cut-off point, the sensitivity, specificity, positive predictive value and negative predictive value of SPA-PSV to identify affected fetuses was 84.4% (32 from 36 cases), 98.1%, 93.1% and 95.3% respectively. Nearly all normal fetuses had a normal SPA-PSV.

Conclusion: SPA-PSV assessment at mid-pregnancy may be used as an adjunct method to identify fetuses with Hb Bart's disease with high, but not perfect, accuracy and may reduce the risk from unnecessary cordocentesis in some fetuses.

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Introduction

Thalassemia is the most common hematologic genetic disease in South East Asia where the carrier rate of α-thal1 trait (– SEA type) is as high as 14% in our population [1], and α-thalassemia is the most common cause of hydrops fetalis, accounting for 80 – 90% of cases [2]. With population migrations during the past decade, this syndrome is now being seen in increasing numbers in other parts of the world. Pregnancies with Hb Bart's disease inevitably result in fetal death during the third trimester or shortly after birth and are frequently associated with serious maternal morbidity, and even mortality, such as pre-eclampsia, dystocia, postpartum hemorrhage due to a large placenta, and the psychological burden for carrying a nonviable fetus to term. Therefore, it is justified to control this condition, especially by prenatal approach [3]. Traditionally, prenatal diagnosis of fetal anemia is based on invasive methods to directly determine fetal hemoglobin through cordocentesis. Both methods pose a risk to the fetus. Amniocentesis is related to a rate of fetal loss of 0.5 – 1% [4, 5] and cordocentesis is associated with a rate of fetal loss of 1.4 – 2.7% [6, 7]. To avoid such complications, researchers have currently attempted to diagnose fetal anemia using ultrasound criteria in fetuses at risk for hydrops fetalis secondary to anemia. It is well-established that the middle cerebral artery peak systolic velocity can effectively identify fetal anemia due to Rh alloimmunization [8 – 11] and possibly Hb Bart's disease. To be a useful test, ultrasound must reliably detect fetal anemia before the onset of overt hydrops fetalis. The fetus can compensate for moderate degrees of anemia by hemodynamic adjustments, but once the hemoglobin deficit exceeds 7 gm/dl, the fetal functional reserve is exhausted with resulting hydropic changes [12]. Likewise, the assessment of splenic artery peak velocity (SPA-PSV) in early studies is useful for predicting fetal anemia secondary to Rh alloimmunization [13 – 15] and possibly secondary to Hb Bart's disease among the fetuses at risk [16]. Our preliminary study showed that the SPA-PSV of affected fetuses was significantly higher than that of unaffected ones [16], suggesting that SPA-PSV assessment at mid-pregnancy may have a potential role in identifying fetuses with Hb Bart's disease among pregnancies at risk. However, the effectiveness of SPA-PSV for differentiating affected from unaffected fetuses among pregnancies at risk has never been evaluated. The objective of this study was to determine the accuracy of SPA-PSV for predicting Hb Bart's disease of fetuses at mid-pregnancy among pregnancies at risk.

Materials and Methods

This study was conducted at a tertiary center, Maharaj Nakorn Chiang Mai Hospital, where the program of prenatal control for severe thalassemia syndrome is well-established [17], with the approval of the research ethics committee. Pregnancies at risk of having fetuses with Hb Bart's disease, of which both of the couples were carriers for alpha-thalassemia1, at 18 – 22 weeks of gestation attending antenatal care clinic unit, between December, 2007 and February, 2009, were recruited for the study. All patients underwent standard ultrasound examination including SPA-PSV assessment before cordocentesis for fetal blood analysis. The inclusion criteria included 1) pregnancies at a gestational age of 18 – 22 weeks, based on accurate last menstrual period or fetal parameters from ultrasound in the first half of pregnancy, 2) pregnancies at risk of having fetuses with Hb Bart's disease, recruited from our thalassemia screening program, and with a carrier status confirmed by PCR (SEA type). The exclusion criteria were 1) fetal anomaly other than hydrops fetalis secondary to Hb Bart's disease, and 2) loss to follow-up or the final diagnosis could not be obtained. Cordocentesis for fetal blood analysis was performed after SPA-PSV measurement. The final fetal diagnoses used as gold standard were based on fetal blood analysis using high-performance liquid chromatography (HPLC) technique by demonstration of unbound Hb (Hb Bart's and Hb Portland), no HbF and no HbA.

All ultrasound examinations were performed with real-time equipment using Voluson E8 (Voluson E8, GE Healthcare, USA), or Aloka SSD alpha-10 (Tokyo, Japan) with transabdominal 3.5 or 5.0 MHz curvilinear transducers. Both machines were similar in term of high resolution and high quality. For examination, the pregnant women were in the semi-recumbent position. All measurements were performed in a period of no fetal breathing and movement. The measurement of SPA-PSV Doppler waveforms was done by the authors as follows (**Fig. 1**): 1) the fetal abdomen was first imaged in an axial view at the level of the stomach by means of real-time ultrasonography with a 3.5 or 5.0 MHz transducer, as appropriate, with color and pulsed Doppler capabilities, 2) color Doppler was used to localize the splenic artery as it arise from the celiac axis and courses behind the stomach into the splenic hilum. 3) Pulsed Doppler evaluation was performed with a sample volume of 2 mm, and an angle of insonation between 0 and 30 degrees from the ultrasound beam, 4) the

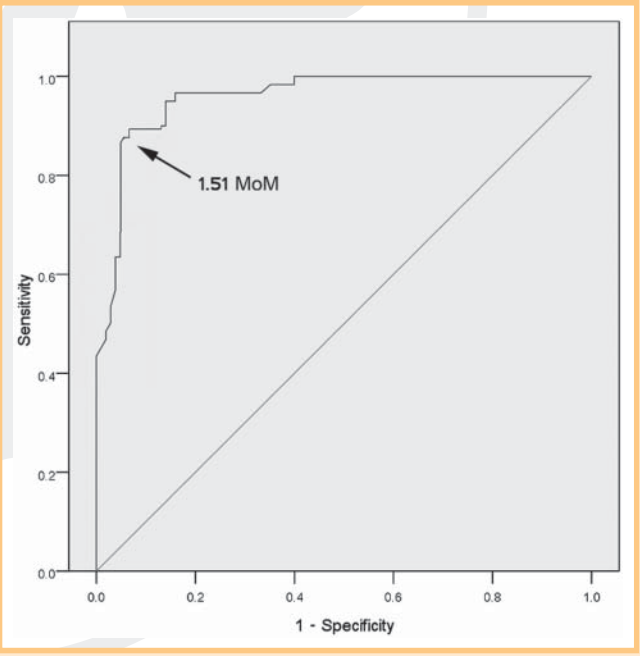

Fig. 1 Color flow of splenic artery, located just behind the stomach and spectral Doppler waveforms of a fetus with Hb Bart's disease at 20 weeks of gestation, depicts high SPA-PSV of 48.19 cm/sec.

Abb. 1 Farbkodierter Fluss in der A. lienalis, die direkt dorsal des Magens lokalisiert ist und Dopplerkurven eines Fetus mit Hb-Bart-Erkrankung in der 20. Schwangerschaftswoche, welche eine hohe SPA-PSV von 48,19 cm/s zeigen.

fetal diagnoses			
SPA-PSV	**hemoglobin Bart's fetuses (n)**	**non-hemoglobin Bart's fetuses (n)**	**total**
> 1.51 MoM	27	2	29
< 1.51 MoM	5	102	107
total	32[1]	104	136

Table 1 Cross-tabulation between result of HPLC and SPA-PSV value.

Sensitivity of SPA-PSV = 84.4 % (95 % CI 71.8 – 97.0), Specificity of SPA-PSV = 98.1 % (95 % CI 95.4 – 100), Positive predictive value = 93.1 % (95 % CI 83.9 – 100), Negative predictive value = 95.3 % (95 % CI 87.6 – 100).

[1] 8 fetuses show some degree of hydropic changes.

sample volume was positioned along the splenic artery in close proximity to its origin from the celiac axis, 5) the peak systolic velocity and lowest diastolic velocity are calculated with electronic calipers, 6) three consecutive waveforms are analyzed and the results are averaged, 7) measurements were obtained during fetal apnea, 8) the above steps were repeated at least three times.

To evaluate the measurements of the SPA-PSV as the multiples of median (MoM) for gestational age, we used a nomogram for various gestational ages derived from a group of 540 normal fetuses between 14 and 40 weeks of gestation [18]. Receiver operator characteristic (ROC) curves were employed to evaluate the relationship of the sensitivity (the true-positive rate) and the false-positive rate (1-specificity) of different threshold values of the SPA-PSV, using the statistical package for the social sciences (SPSS) version 17.0 (Chicago, USA). An abnormal increase in SPA-PSV was defined as the value above the optimal cut-off level (MoM) of each gestational age, derived from the ROC curves. The main outcome measure was the sensitivity, specificity, positive predictive value, and negative predictive value of SPA-PSV in identifying fetuses with Hb Bart's disease, using the optimal threshold value (MoM) based on ROC curves. The conduction of this study was approved by the ethics committee and supported by the Thailand Research Fund (TRF).

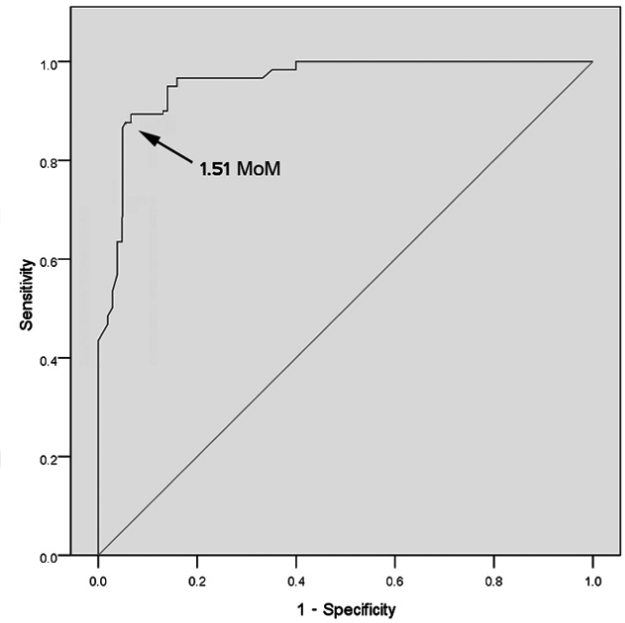

Fig. 2 Receiver operating characteristic (ROC) curve of SPA-PSV for differentiating normal fetus from fetuses with Hb Bart's disease.

Abb. 2 ROC(Receiver Operating Characteristic)-Kurve der SPA-PSV zur Differenzierung von gesunden Feten und solchen mit Hb-Bart-Erkrankung.

Results

During the study period (December 2007–February 2009), 132 singleton pregnancies and 2 twin pregnancies at risk of Hb Bart's disease were sonographically measured for SPA-PSV and underwent cordocentesis for fetal blood analysis during 18 – 22 weeks of gestation. Of 134 recruited pregnant women, the mean maternal age was 28.7 ± 5.4 years (range 18 – 44 years) and the mean gestational age was 19.1 ± 1.02 weeks (range 18 – 22 weeks). Thirty-six fetuses with Hb Bart's disease were finally diagnosed by fetal blood analysis with high performance liquid chromatography (HPLC) and the remainders were normal or a-thal-1 trait, defined as unaffected fetuses. The mean SPA-PSV (± SD) of the normal pregnancies (fetuses with normal a-globin genes or a-thal1 trait) and pregnancies with fetal Hb Bart's were significantly different, 19.2 ± 3.1 (range from 12.7 – 25.9) and 25.5 ± 4.5, (range from 20.4 – 34.8) respectively (Student's t-test; p-value < 0.001). According to the ROC curves (**Fig. 2**), the optimal SPA-PSV threshold value was 1.51 MoM for each gestational age for predicting Hb Bart's disease. If using SPA-PSV above 1.51 Multiple of Median (MoM) as a cut-off point, the sensitivity, specificity, positive predictive value and negative predictive value of SPA-PSV for predicting affected fetuses was 84.4% (32 from 36 cases), 98.1%, 93.1% and 95.3% respectively, as shown in the **Table 1**. Nearly all normal fetuses (102 from 104) had normal SPA-PSV.

Of 32 fetuses with Hb Bart's disease, eight of them (15.6%) had some sonographic signs of early hydrops fetalis, such as pleural effusion or ascites or subcutaneous edema. All of them had high SPA-PSV without overlapping with that of normal fetuses for each gestational week.

Discussion

Since splenic artery circulation is sensitive to both red blood cell destruction and production, changes in splenic artery circulation, as assessed by Doppler velocimetry, are likely to correlate with the degree of fetal anemia. SPA-PSV has been shown to correlate closely with a degree of fetal hemoglobin [14]. Fetuses with Hb Bart's disease are associated with hyperdynamic circulation and low blood viscosity secondary to anemia, resulting in an elevated splenic artery peak systolic velocity in several vessels as we observed in the middle cerebral artery [19] as well as in the splenic artery [16]. We postulated that SPA-PSV should be increased in fetuses with Hb Bart's

disease due to anemia in as early as mid-pregnancy while the hydropic change is subtle and it may be useful in clinical application.

In addition to confirmation of our previous observation of a strong correlation between SPV-PSV and fetal Hb Bart's disease[16], this study demonstrates the potential role of clinical application with high accuracy for differentiating affected from unaffected fetuses among pregnancies at risk. However, the accuracy of SPA-PSV for predicting Hb Bart's disease may not be as high as that reported for predicting fetal anemia in fetuses with Rh isoimmunization [14, 15]. This may be due to the fact that, although all fetuses with Hb Bart's disease had some degree of anemia, some affected fetuses at mid-pregnancy may not have very low hemoglobin but functional anemia secondary to ineffective hemoglobin [20]. These fetuses had no true anemia and no decreased blood viscosity resulting in no increase peak-systolic velocity in peripheral vessels, leading to a false-negative test. Therefore, SPA-PSV of some affected fetuses was overlapped with that of some unaffected ones. However, it seems to be useful for identifying the proper candidates for cordocentesis. For example, a fetus with normal SPA-PSV has little chance of Hb Bart's disease and cordocentesis may be obviated and serial ultrasound may be preferred. Technically, SPA-PSV can modify the risk of the fetuses from 25% to be near zero or more than 90%. This information can help couples to select the method of further prenatal diagnosis. For example, if normal SPA-PSV is demonstrated, the risk may probably be changed to almost zero, serial ultrasound to detect early signs of hydrops fetalis may probably be a good choice, and invasive diagnostic procedure can be obviated or can be reserved for when the early sonographic signs of fetal anemia appear. However, a high SPA-PSV among fetuses at risk of Hb Bart's disease at mid-pregnancy is highly suggestive, but not absolutely diagnostic, of an affected pregnancy, for which an invasive diagnostic procedure is strongly indicated. Moreover, it may be used as an adjunct to other ultrasound markers for fetal Hb Bart's disease, such as cardiothoracic ratio or middle cerebral artery. Since the main objective of prenatal diagnosis of Hb Bart's disease is the reduction of maternal morbidity rather than the prevention of the birth of affected infants, serial measurements of SPA-PSV may be an alternative cost-effective method for the exclusion of the disorder, particularly in areas where resources for prenatal diagnosis are limited. In our population, the main prenatal diagnostic technique is cordocentesis at 18 to 22 weeks, and it is performed only in tertiary centers. It is very important for community hospitals to select only cases at high risk for Hb Bart's disease for referral for invasive prenatal diagnosis. Therefore, the measurement of SPA-PSV may be an alternative cost-effective method for identifying cases for referral or follow-up with serial ultrasonography in their own hospitals. With this approach, invasive procedures can be performed selectively, and fewer fetuses will be lost unnecessarily. The reduction in medical expenses and procedure-related fetal loss is likely to be substantial. Although a few affected fetuses may be missed by this approach, these cases can be suspected or detected by later serial ultrasonography. Moreover, since the disease is uniformly lethal and termination of pregnancy can be offered any time when the diagnosis is made reliably, missing it in a very small number of cases will not greatly influence the overall outcome. Despite the high accuracy of SPA-PSV, the diagnosis should be confirmed with definitive methods, such as fetal blood analysis, to exclude false-positive results, even though the rate is low, and hydrops fetalis secondary to other causes. Compared to other sonographic markers for Hb Bart's disease at mid-pregnancy, SPA-PSV seems to be somewhat less predictive than MCA-PSV [21] and cardiothoracic ratio [22]. However, further studies to compare these markers in the same setting should be done and see if they have an additive effect on accuracy or not.

Several limitations should be mentioned regarding this study. Since we did not determine the fetal hemoglobin levels, we could not correlate the degree of anemia and SPA-PSV. However, this is not a problem in clinical application since, unlike the assessment of fetal Rh isoimmunization, we just need to know whether the fetus is affected or not in an all-or-none fashion, not the degree of fetal anemia. Another limitation is that the results presented here can properly be applied only to fetuses at risk (25% having an affected fetus), not to the general population with a low prevalence of Hb Bart's disease. Additionally, SPA-PSV measurements require training, like MCA-PSV. Thus, it may not always be practical in general practice.

Eight of 32 fetuses with Hb Bart's disease in this series were associated with early hydropic changes, signifying severe anemia. Among these fetuses, SPA-PSV may be of little clinical value because other hydropic signs warrant further work-up without the need of SPA-PSV. The future study should focus on fetuses in early pregnancy without hydropic signs to see if SPA-PSV can differentiate affected from unaffected ones before sonographic signs of hydrops fetalis become evident.

There may have been a bias in this study. This was due to the fact that Doppler evaluation of the splenic artery was not a blind method since the examiner knew the morphology of the hydropic changes from conventional sonographic images, especially in the cases in which hydrops fetalis had already occurred at the time of examination. Therefore, the diagnosis of Hb Bart's disease or high SPA-PSV could have been anticipated. The strength of this study includes a large sample size for gaining power of test. Additionally, the nomogram used for SPA-PSV evaluation was derived from our own population.

In conclusion, this study suggests that SPA-PSV assessment can provide additional information regarding the risk of having fetal Hb Bart's disease and may help couples to make a decision about the technique of further fetal diagnosis, either invasive diagnosis with cordocentesis or serial ultrasound. Moreover, this new marker may be used as an adjunct to other ultrasound markers for fetal Hb Bart's disease, such as cardiothoracic ratio or middle cerebral artery.

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Normal Length of the Fetal Liver from 14 to 40 Weeks of Gestational Age

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ABSTRACT: *Objective.* To create a normal reference table of fetal liver length in normal fetuses

Methods. A prospective, cross-sectional study was conducted on normal pregnancies with accurate gestational age (GA) from 14 to 40 weeks of gestation. All fetuses were measured for fetal liver length from the top of the right hemidiaphragm to tip of the right liver lobe on coronal image of the fetal abdomen, using high-resolution real-time ultrasound with a 2- to 4-MHz convex transducer.

Results. A total of 685 normal pregnant women between 14 and 40 weeks of gestation were recruited. Forty-five were excluded due to poor image quality and fetal abnormalities. The remaining 640 were available for analysis. The linear regression model was best fitted to estimate the 5th, 50th, and 95th percentile range of liver length at each gestational week. Fetal liver length was gradually increased with GA with fitted equation as follows: Liver length (mm) = 1.61 (GA, week) − 6.75 ($r^2 = 0.94$, $p < 0.001$).

Conclusion. A normal reference range of fetal liver length for each GA between 14 and 40 weeks was constructed. This may be a useful tool in assessment for some fetal pathologic conditions, especially when fetal anemia is suspected. © 2010 Wiley Periodicals, Inc. *J Clin Ultrasound* **39**:74–77, 2011; Published online in Wiley Online Library (wileyonlinelibrary.com). DOI: 10.1002/jcu.20756

Keywords: fetus; liver length; nomogram; ultrasound; obstetrics

The fetal liver is an active site of hematopoiesis in the second trimester and it is a major site of extramedullary hematopoiesis in fetuses complicated by anemia such as Rh isoimmunization, resulting in enlargement of liver size.[1] In addition, liver size is markedly affected by abnormal fetal growth. Therefore, measurement of fetal liver length can be helpful in the diagnosis of several pathologic conditions associated with changes in fetal liver size, for example, a decrease in liver size associated with intrauterine growth restriction[2] or liver enlargement associated with fetal anemia due to severe Rh isoimmunization[1] or Hb Bart's disease[3] or hepatomegaly in the case of maternal diabetes.[4] However, it is essential that normal reference range of liver length be first established. A number of reports have indicated that the average birth weight in Eastern populations is lower than in Western populations and it has been shown that different standards of ultrasound-based fetal growth are needed for different populations.[5,6] Therefore, nomograms of the fetal liver length may be population-specific.

Although nomograms of liver length have been previously published, most are based on small sample size and/or do not include fetuses in early second trimester, which is particularly helpful in the prediction of severe anemia occurring in early gestation such as in Hb Bart's disease.[7,8] Moreover, some did not provide a table of predictive values for each gestational age.[9] Also, the lower border of the liver is often unclear and not easily distinguished from surrounding structures using earlier models ultrasound machines.

The objective of the present study was to establish the nomogram of fetal liver length in a Thai population with a high-resolution machine and adequate sample size from 14 to 40 weeks of gestation.

MATERIALS AND METHODS

This prospective cross-sectional descriptive study was conducted between September 1st,

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2007 and October 31st, 2009 in the Department of Obstetrics and Gynecology, Faculty of Medicine, Chiang Mai University, Thailand. This research was approved by the research ethical committee of our institution and financially supported by the Thailand Research Fund and the Commission on Higher Education of Thailand. Pregnant women attending our antenatal care clinic were recruited into the study and signed written informed consent. Inclusion criteria included an accurate gestational age (GA) based on regular menstruation with knowledge of the exact date of last menstrual period and fetal biometry in the first half of pregnancy, a GA between 14 and 40 weeks, and a low-risk pregnancy without any obstetrical or medical complication. Exclusion criteria included fetal malformations or chromosome abnormalities, small-for-gestational age fetuses (less than 10th percentile for a given GA), or macrosomia (more than 90th percentile), multifetal pregnancy, and poor imaging of fetal liver border on ultrasound examination.

All ultrasound examinations were performed by experienced perinatologists using Voluson E8 scanner (GE Healthcare, Milwaukee, WI) with a transabdominal 2- to 4-MHz curvilinear transducer. In each case, a routine standard sonographic examination, including fetal anomaly screening and fetal biometry, was performed first. The steps in liver length measurement were as follows: the fetal upper abdomen was first imaged in an axial view at the level of the stomach and the liver was demonstrated to occupy the most part of the fetal abdomen. To obtain the proper plane for measurement, the transducer was swiveled and the aorta was imaged in the longitudinal plane. The transducer was then moved parallel to this plane until the tip of the right lobe and right hemidiaphragm were demonstrated. Occasionally, the transducer had to be slightly rotated or tilted to visualize both the diaphragm and the tip of the right lobe of the liver. Fetal liver length was measured from the top of the right hemidiaphragm, usually at the right margin of the heart in contact with the hemidiaphragm, to the tip of the right lobe using the electronic calipers of the scanner as shown in Figure 1. At least three sets of measurements were made and the values were averaged and recorded. Baseline demographic and obstetric data of the pregnant women were also recorded. The ultrasound examinations were performed by two operators. The first 20 measurements were performed by both of them to assess interobserver variations.

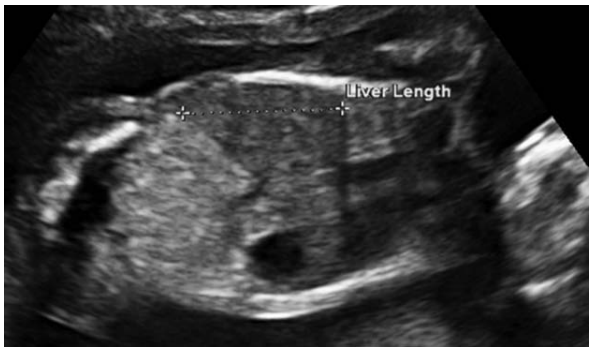

FIGURE 1. Liver length measurement of a 18-week fetus in the right-side-up position.

Statistical Analysis

GA as an independent variable and liver length as a dependent variable were used to generate the best-fit regression equation, using the SPSS software version 17.0 (SPSS, Chicago, IL). The resulting residuals were tested for normality of distribution, using Kolmogorov-Smirnov and Shapiro-Wilk tests. If necessary, the data were transformed and the new residuals were checked if they conformed to a normal distribution. Data were also examined to determine whether the standard deviation of the residuals varied across the range of values for GA using Levine's test. If a significant heteroscedasticity was found, weighted regression of absolute residuals was used to adjust the standard deviation. Based on the best-fit equation and the predicted standard deviation, predictive values for 5th, 50th, and 95 percentile ranges of fetal liver length were constructed.

RESULTS

A total of 685 pregnant women were recruited into the study and 45 were later excluded due to poor quality ultrasound images, especially when the fetuses were in right-side-down position, fetuses with growth restriction (birth weight of less than 10th percentile), macrosomia, and fetal structural or chromosomal anomalies, including congenital heart defects,[5] abdominal wall defect,[3] and diaphragmatic hernia.[1] In the remaining 640 women, the average number of measurements per week was 20.7, ranging from 12 to 30 per week. The mean (± SD) maternal age was 27.4 ± 5.7 years (range, 15–43 years). Two-hundred ninety-four (45.9%) women were nulliparous. The liver length was found to increase in a linear fashion throughout the second and third trimesters (Figure 2). The liver length had normal

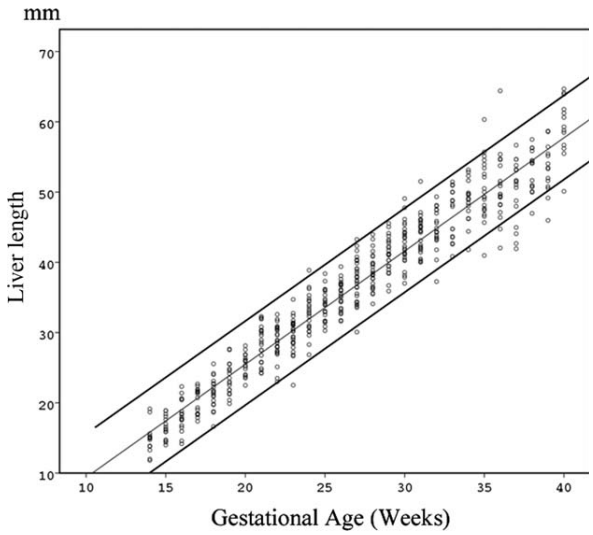

FIGURE 2. Scattergram of the liver length by gestational age. The three lines represent predicted value of mean and 95% confidence intervals.

distribution at all GAs. The best regression model was found to be linear with the following best fitted equation: Liver length (mm) = 1.61 GA (in weeks) − 6.750 ($r^2 = 0.94$, $p < 0.001$). Values of liver length at each GA for 5th, 50th, and 95th percentiles are shown in Table 1. The interobserver mean difference for the measurement of liver length was 4.6%.

TABLE 1
Nomogram of Predicted Liver Length Values (mm) by Gestational Age Shows 5th, 50th, and 95th Percentiles

	Fetal Liver Length (mm) Percentiles (Smoothed)		
GA (weeks)	5th Percentile	50th Percentile	95th Percentile
14	9.9	15.8	21.7
15	11.6	17.4	23.3
16	13.2	19.0	24.9
17	14.8	20.6	26.5
18	16.4	22.3	28.1
19	18.0	23.9	29.7
20	19.6	25.5	31.3
21	21.3	27.1	32.9
22	22.9	28.7	34.6
23	24.5	30.3	36.2
24	26.1	31.9	37.8
25	27.7	33.5	39.4
26	29.3	35.2	41.0
27	30.9	36.8	42.6
28	32.5	38.4	44.2
29	34.2	40.0	45.8
30	35.8	41.6	47.5
31	37.4	43.2	49.1
32	39.0	44.8	50.7
33	40.6	46.4	52.3
34	42.2	48.0	53.9
35	43.8	49.7	55.5
36	45.4	51.3	57.1
37	47.0	52.9	58.7
38	48.6	54.5	60.4
39	50.3	56.1	62.0
40	51.9	57.7	63.6

DISCUSSION

This study has shown that fetal liver length in normal Thai fetuses gradually increases with linear growth pattern from 14 to 40 weeks of gestation. In general, the fetal liver is the earliest and most severely affected organ in fetal growth abnormalities. The direct ultrasonographic measurement of the fetal liver, therefore, may be helpful in evaluating early stages of abnormal fetal growth. We believe that this normative data of fetal liver length may have several applications in clinical practice such as in evaluation of fetal growth in cases of intrauterine growth restriction,[2] gestational diabetes,[4] twin-to-twin transfusion syndrome,[10] and in surveillance of fetal anemia due to isoimmunization[1,11,12] or hemoglobin Bart's disease.[13]

Compared with the normal reference range in previous reports between 20 and 41 weeks of gestation,[14–16] fetal liver length in our study is slightly lower. This may be due to racial factors. This finding suggests the need to construct separate nomograms for specific populations. Although our data seem to be comparable to those reported by Phatihattakorn et al in the Thai population,[9,15,16] these authors derived their nomogram from raw data, not from the fitted regression equation. Due to a possible racial effect, our nomogram may be more appropriate for Eastern than Western populations.

Liver size may be useful in differentiating anemic fetuses from nonanemic ones among those at risk for severe anemia, especially Hb Bart's disease. Recently, the liver volume has also been studied.[17–19] However, liver volume measurement from 3D datasets is time-consuming and rarely used in daily practice. We believe that a simple measurement of fetal liver length may be more practical. Our study also showed good reproducibility with a low interobserver variation.

Because anemia in Hb Bart's disease, which is a common problem in Southeast Asia, usually occurs in early gestation, hepatomegaly in fetuses with Hb Bart's disease may develop as early as in the late first trimester. Therefore it is important that the nomogram includes fetuses in early second trimester. Hepatomegaly is obvious in advanced hydrops fetalis secondary to fetal anemia.[3] However, although the classic hydropic changes due to Hb Bart's disease are likely to occur after 20 weeks, our experience of prenatal

diagnosis in the early second trimester suggests that fetal liver size may be useful in differentiating normal from affected pregnancies. This is yet to be tested in further studies. To the best of our knowledge, no studies regarding liver size as a predictor of Hb Bart's disease in the first half of pregnancy has been reported. Some studies have shown that the liver length could identify affected pregnancies with a high sensitivity and specificity in fetuses with Rh isoimmunization, although they included few patients at 18–22 weeks of GA.[1,20]

A limitation of our study is that, in several fetuses in right-side-down position, the liver could not be clearly delineated, resulting in exclusion of those cases. This suggests the inability to apply this measurement to all fetuses.

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IMAGING

Venous Doppler studies in low-output and high-output hydrops fetalis

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OBJECTIVE: The objective of the study was to compare fetal venous Doppler flow reflecting cardiac function in fetuses with hydrops fetalis between a group of congenital heart defect (low cardiac output) and a fetal anemia group (high cardiac output).

STUDY DESIGN: This was a prospective cross-sectional analysis. It was conducted at the Maharaj Nakorn Chiang Mai Hospital, Tertiary center, Medical School. The study included fetuses with hydrops fetalis secondary to cardiac causes (low output group) and anemia (high output group). All fetuses underwent ultrasound examination to assess ductus venosus (DV) and umbilical vein (UV) Doppler indices. The results were related to normal reference range and were also compared between the group of high-output and the low-output group.

RESULTS: Sixty-nine hydropic fetuses were available for analysis, 50 in the high-output group and 19 in the low-output group. The peak velocity index, preload index, and the pulsatility index of the DV were significantly low in the high-output group, whereas they were significantly high in the low-output group. The umbilical vein pulsations were found in 78.9% of the fetuses with low-output hydrops fetalis but only 28.0% of fetuses in the high output group ($P < .001$).

CONCLUSION: New insights gained from this study are that hydrops caused by severe anemia because of hemoglobin Bart's is not associated with high central venous pressures as is seen in hydropic fetuses with coronary heart disease. This suggests that cardiac decompensation is not the primary mechanism of hydrops in these anemic fetuses. Additionally, umbilical vein pulsations are not a sign of cardiac failure in the anemic group.

Key words: cardiac output, Doppler velocity, ductus venosus, hydrops fetalis

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Fetal hydrops represents a specific condition characterized by an increase of total body water content, defined by fluid collections in at least 2 fetal body compartments such as ascites, pleural, and/or pericardial effusion and/or the skin edema. Hydrops fetalis is not a diagnosis in itself but a symptom and the end-stage of a wide variety of disorders. In chromosomally normal fetuses, low-output (myocardial dysfunction) and high-output (anemia) hydrops fetalis account for the large portion of cases.[1] Based on the largest systematic review by Bellini et al,[1] congenital heart defect is a major cause of hydrops fetalis, accounting for 21.7%. However, in Southeast Asia hemoglobin Bart's disease is responsible for 70-85% of all cases.[2]

Pathophysiology of hydrops fetalis remains controversial. The postulated mechanisms are cardiac failure secondary to either cardiac defects (low cardiac output) or fetal anemia (high cardiac output). Additionally, other causes such as decreased colloid oncotic plasma pressure or obstruction of venous/lymphatic flow can play a role in a minority of cases.

In fetal anemia, increased cardiac output seen in these fetuses is believed to be due to a decrease in blood viscosity, which, in turn, leads to increased venous return and cardiac preload, resulting in cardiac failure and finally hydrops fetalis. However, Hecher et al[3] showed that fetal anemia in nonhydropic Rh isoimmunized fetuses is associated with a hyperdynamic circulation in both arterial and venous vessels, but even in severe anemia, there is no evidence of congestive heart failure. This is consistent with our experience in fetal anemia caused by hemoglobin Bart's disease.

We have often observed a normal cardiac function in these fetuses with frank hydrops fetalis, raising a question of cardiac failure as a primary cause of hydrops fetalis because of anemia. Therefore, this study focused on venous Doppler changes, as a surrogate marker for cardiac function assessment, in fetal hydrops secondary to low cardiac output (congenital heart disease) and high cardiac output (anemia).

There have been several studies on arterial Doppler changes in fetal anemia, but very few publications have studied on venous Doppler change, especially when hydrops fetalis has already developed. Because venous Doppler changes directly reflect cardiac decompensation, the studies may be informative for pathophysiology of hydrops fetalis. Understandings on venous Doppler changes may be helpful in

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predicting prognosis or differentiating causes or making a choice of management. Moreover, the difference between cardiac function in low-output and high-output hydrops fetalis has never been published.

The purpose of this study was to compare fetal venous Doppler indices among fetuses with hydrops fetalis between a group of congenital heart defect (low cardiac output) and a fetal anemia one (high cardiac output).

Materials and Methods

A cross-sectional prospective analytic study was conducted with the approval of the Research Ethics Committee 3, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand. Singleton pregnancies with hydropic fetuses secondary to hemoglobin Bart's disease and those with cardiac causes were recruited into the study with informed written consent.

The prenatal diagnosis of hydrops fetalis was defined by demonstration of fluid accumulations in at least 2 fetal body compartments such as serous cavities of the fetus (abdominal ascites, pleural, and/or pericardial effusion) and/or the skin edema. When hydrops fetalis was diagnosed, detailed ultrasound was performed and causes of hydrops fetalis were identified. Hydrops fetalis due to causes other than hemoglobin Bart's and congenital heart defects was excluded from analysis.

The diagnosis of hemoglobin Bart's disease was based on fetal cord blood analysis, whereas the diagnosis of congenital heart diseases was based on detailed fetal echocardiography by an experienced examiner. Ultrasound examinations were performed using real-time machines either Aloka alpha-10 (Tokyo, Japan) or Voluson E8 (GE Healthcare, Indianapolis, IN). The maximal ultrasound intensity was set at 100 mV/cm^2 spatial peak temporal average and the high-frequency filter set at 125 Hz to remove signals from slow moving tissues. On ultrasound examination, venous Doppler waveforms were recorded from the ductus venosus and the umbilical vein at a free-floating loop of the cord. The size of the sample volume was adapted to the vessel diameter to cover it entirely.

All recordings used for measurements were obtained in the absence of fetal breathing movements, and the angle between the ultrasound beam and the direction of blood flow was less than 30°, and velocity measurements were taken after correction for the angle of insonation. The best 3 consecutive waveforms were automatically analyzed by the built-in software.

The venous Doppler indices examined included the peak forward velocity during systole (S), representing forward flow secondary to dilatation of right atrium during ventricular systole, peak forward velocity during diastole (D), reflecting ventricle filling during diastole, and lowest forward velocity or peak reversed velocity during atrial contraction (a) as well as the time average maximum velocity (Tamx) were determined. The peak velocity in veins (PVIV; S-a/D), preload index (PLI; S-a/S), and the pulsatility index for veins (PIV; S-a/Tamx) were automatically calculated during the measurement.

Umbilical venous blood velocity was recorded. Pulsating blood velocity in the umbilical vein was defined as a decrease in velocity by more than 15% of the maximal velocity. When more than 1 ultrasound examinations were performed, only the last one was included to the analysis. The results were related to normal reference range data of venous Doppler for the ductus venosus.[4] The data were also compared between the group of high-output hydrops fetalis and the low-output group.

Statistical analysis was performed with SPSS version 17 for Windows (SPSS, Inc, Chicago, IL). Student *t* test or Mann-Whitney *U* test were used for the comparison of continuous variables. The Kruskal-Wallis nonparametric test was used to evaluate trends in z-score or mean difference. Comparison of proportion was performed with χ^2 or Fisher's exact test where appropriate. $P < .05$ was considered statistically significant.

TABLE 1
Distribution of the causes of hydrops fetalis

Causes	n
High-output hydrops fetalis	50
Hemoglobin Bart's disease	50
Low-output hydrops fetalis	19
Ebstein's anomaly	5
Hypoplasia left heart	4
Atrial flutter/supraventricular tachycardia	3
Heterotaxy	3
Small heart syndrome	3
Tricuspid atresia	1

Tongsong. Venous Doppler in hydrops fetalis. Am J Obstet Gynecol 2010.

Results

Sixty-nine singleton pregnancies with fetal hydrops were successfully recorded for venous Doppler waveforms and available for analysis. Of these, 50 fetuses had hydropic changes due to hemoglobin Bart's disease, classified as the high-output hydrops fetalis and 19 were hydrops fetalis secondary to congenital heart defect, classified as the low-output hydrops fetalis. The causes of hydrops fetalis are summarized and presented in Table 1. The mean gestational age was 26.3 ± 5.2 weeks (range, 17–36 weeks), 27.7 ± 5.2 weeks, and 25.7 ± 3.7 weeks for all fetuses, the high-output group, and the low-output group, respectively.

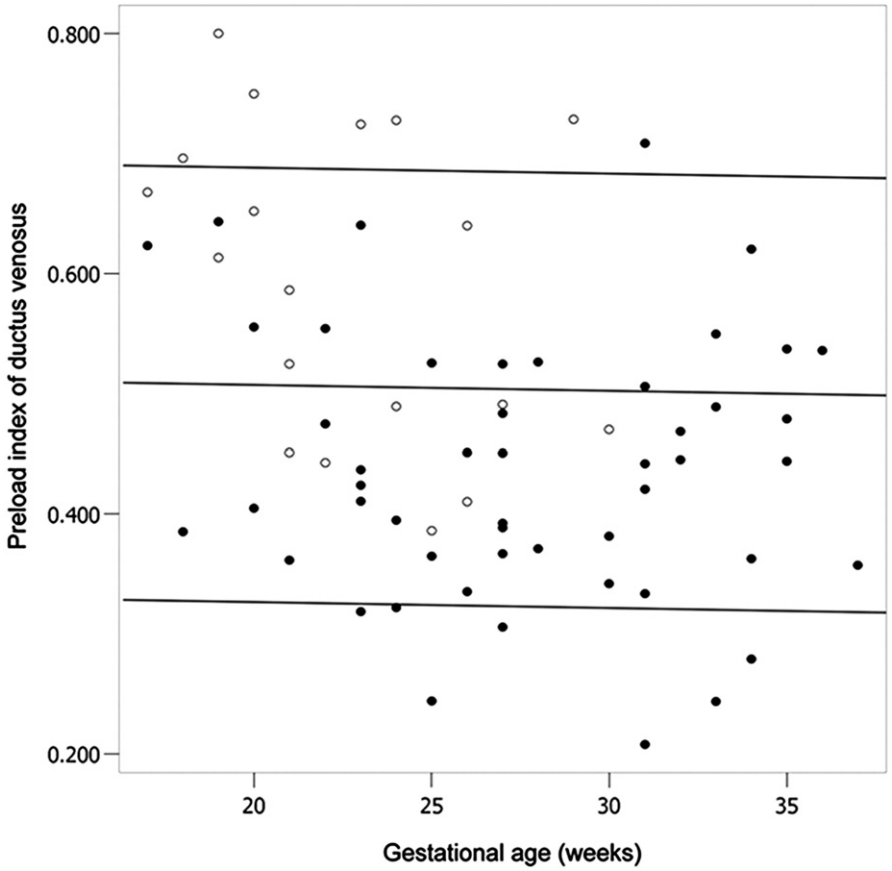
Venous Doppler studies show that most fetuses in both groups had significantly difference in ductus venosus indices, in terms of PLI, PVIV, and PIV. When compared with the normal values of the reference ranges reported by Baschat,[4] the mean differences from normal mean of the Doppler indices in the high-output group were significantly lower (Student *t* test; $P < .001$), whereas the mean differences of the Doppler indices in the low-output group were significantly higher than those in normal mean for each gestational week (Student *t* test; $P < .001$) (Table 2). The scattergram of PLI, PVIV, and PIV of the ductus venosus is presented in Figure 1.

TABLE 2
Comparison of ductus venosus Doppler indices of hydropic fetuses to appropriate normal mean for gestation

Variable	n	Mean difference	Percent change	*t*	*P* value
High-output hydrops fetalis					
Preload index	50	−0.066	−13.16%	−5.045	< .001
Peak velocity index	50	−0.063	−10.86%	−4.224	< .001
Pulsatility index	50	−0.045	−7.018%	−2.489	< .001
Low-output hydrops fetalis					
Preload index	19	0.089	+17.03%	5.746	< .001
Peak velocity index	19	0.149	+25.40%	9.532	< .001
Pulsatility index	19	0.135	+20.54%	5.672	< .001

Tongsong. Venous Doppler in hydrops fetalis. Am J Obstet Gynecol 2010.

FIGURE 1
Preload index of ductus venous in hydrops fetalis



Scatterplot of preload index of high-output (*solid circle*) and low-output (*opened circle*) hydrops fetalis.
Tongsong. Venous Doppler in hydrops fetalis. Am J Obstet Gynecol 2010.

The umbilical vein pulsations were found in most cases of the fetuses with low-output hydrops fetalis (Figure 2), whereas only a small portion of fetuses in the high-output group had umbilical vein pulsations, either 1-phase pulsations (Figure 3) or 2-phase pulsations (Figure 4). The prevalence of the umbilical vein pulsations of the both groups is significantly different (Pearson χ^2 test; $P < .001$) (Table 3).

Of interest, 2 fetuses in the high-output groups showed no umbilical vein pulsations at 20 and 21 weeks but showed 1-phase pulsation the following week, during waiting for final diagnosis from fetal blood analysis. Of note, no case in this study was found to have a triple pulsation of the umbilical vein.

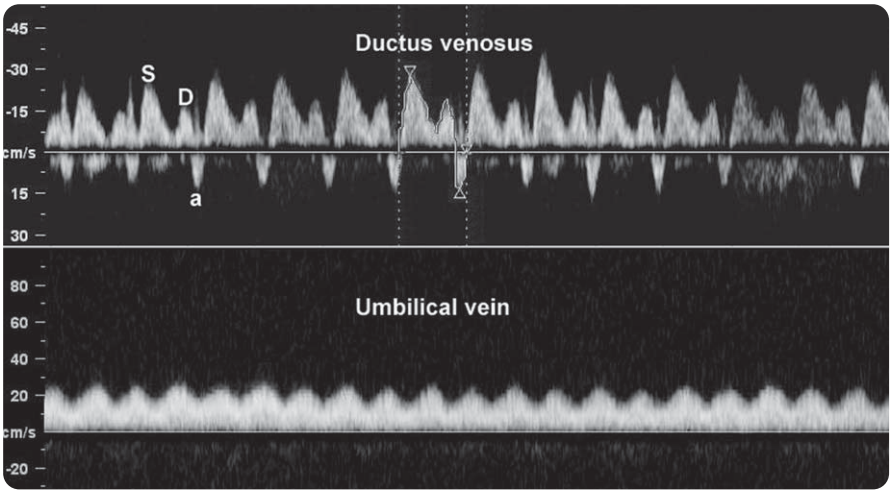
COMMENT

Several fetal conditions such as congenital heart defects, anemia, or infection, leading to myocardial dysfunction, cardiac decompensation, and hydrops fetalis may also correspond with changes in the venous blood flow,[5-8] and venous Doppler studies can therefore be helpful in differentiating causes of hydrops fetalis.

Our results showed the different patterns of venous Doppler between a group of hydrops fetalis associated with low cardiac output (cardiac defects) and a group of high cardiac output (fetal anemia). Low-output hydropic fetuses demonstrated Doppler signs of an increase in central venous pressure in most cases, whereas high-output hydropic fetuses did not show high central pressure reflected by normal or even low preload index, peak velocity index, and pulsatility index for vein in ductus venosus, in spite of hyperdynamic circulation or hypervolemia, indicating hydrops fetalis has developed before cardiac decompensation. This finding is against a long-believed concept that hydrops fetalis is a consequence of congestive heart failure as a result of anemia.

Most fetuses with cardiac defects (ie, Ebstein's anomaly, tricuspid atresia, or hypoplastic heart) have impaired ventricular filling with alteration of the fetal hemodynamics and usually manifest as

FIGURE 2
Example of low-output hydrops fetalis



The *upper panel* shows ductus venosus Doppler waveforms with retrograde flow during atrial contraction. The *lower panel* shows umbilical vein 2-phase pulsations (2 pulses in 1 cardiac cycle). Note that the preload index tends to decrease with gestational age.

Tongsong. Venous Doppler in hydrops fetalis. Am J Obstet Gynecol 2010.

an abnormal venous Doppler signal, indicating increased central venous pressure.[9] Increased pulsatility or preload index in these fetuses may be an early indicator for the occurrence of congestive heart failure followed by fetal hydrops. This may be due to a relative restriction of the foramen ovale because of a massive increase in transatrial right-to-left shunt. In these cases, the ability to increase left ventricular volume flow is mandatory to compensate for the decreased right ventricular function.[9,10]

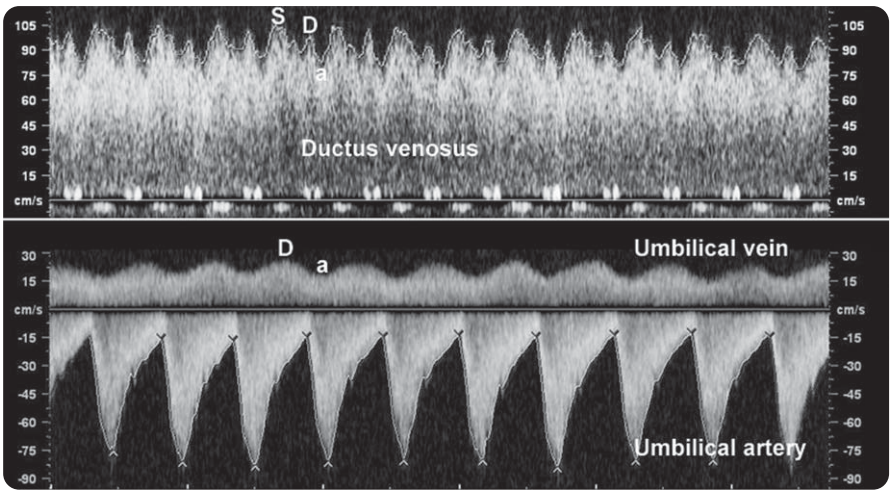
These fetuses are often associated with right atrial and venous pressure increase with subsequent fetal hydrops. Therefore, in the low-output group, abnormal venous Doppler should be interpreted as a sign of cardiac decompensation rather than a primarily hemodynamic change resulting from the cardiac defect. On the other hand, fetuses with severe heart defects with normal venous Doppler indices are unlikely to develop hydropic changes. However, fetuses with cardiac defects with hydrops fetalis almost always show abnormal venous Doppler velocity. The a wave of DV corresponds to atrial contraction, and it was found to be reversed in cases of severe congestive heart failure, probably as a result of an abnormally high atrial pressure,[11] as seen in the low-output group of this study (Figure 2).

Fetal heart failure or hypoxia in growth-restricted fetuses can result in increased central venous pressure and increased pulsatility, leading to the opening of the ductus venosus, propagation of pulsating pattern to the umbilical vein.[5,12-15] Additionally, increased right atrial pressure results in decreased diastolic blood flow velocities and often augments reversal of flow in end-diastole at the time of atrial contraction. This phenomenon was also consistently seen in a group with cardiac defect in this study but not in the anemic group.

Normal umbilical venous flow has no fluctuation due to the filtering effect of the ductus venosus which act as a sphincter-like mechanism that can regulate the amount of oxygenated blood shunted to the foramen ovale.[16] However, increased central venous pressure can facilitate opening of the ductus venosus and thus transmission of central venous pulsations into the umbilical vein. The pattern of umbilical pulsation depends on the degree of its opening.[14,17,18]

Surprisingly, our study indicates that fetal anemia or hypervolemia is not associated with venous Doppler signs of increased central pressure. Most of these fetuses showed normal or even low preload and pulsatility index, suggesting

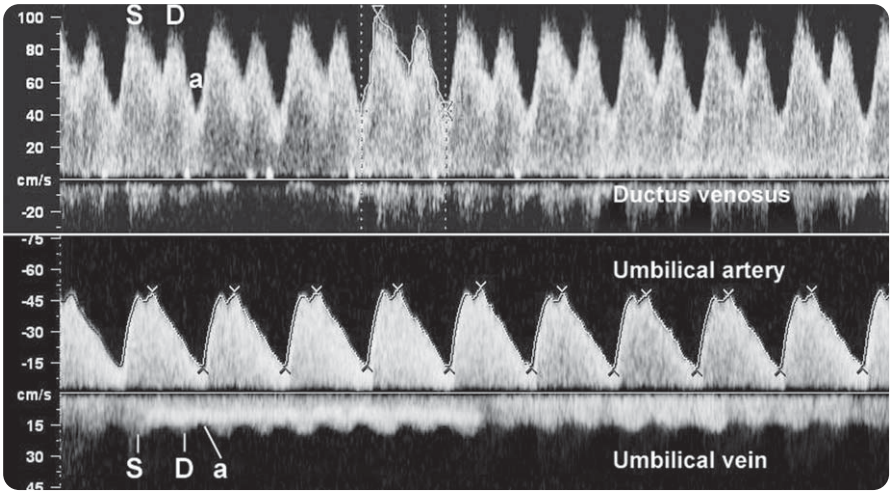
FIGURE 3
Example of high-output hydrops fetalis



The *upper panel* shows ductus venosus Doppler waveforms with normal triphasic pattern with very high forward flow during atrial contraction. The *lower panel* shows umbilical vein pulsations (simultaneously recorded for umbilical artery waveforms) as well as high peak velocity in umbilical artery. Note that the venous pattern shows 1-phase pulsations, representing forward flow during diastolic filling or corresponding to the D wave of ductus venosus, but the S wave does not appear on the umbilical vein recording.

Tongsong. Venous Doppler in hydrops fetalis. Am J Obstet Gynecol 2010.

FIGURE 4
Example of high-output hydrops fetalis



The *upper panel* shows ductus venosus Doppler waveforms with normal pattern with normal preload. The *lower panel* shows umbilical vein pulsations along with umbilical artery recording. Note that the venous pattern shows 2-phase pulsations, representing forward flow during systole (S wave) and diastolic filling (D wave) on the umbilical vein recording.

Tongsong. Venous Doppler in hydrops fetalis. Am J Obstet Gynecol 2010.

that hydrops fetalis is not primarily caused by high-output cardiac decompensation. Although long-lasting hypervolemia can lead to marked cardiomegaly, and finally to cardiac failure,[19] cardiomegaly reflects rather high competency of cardiac compensation than cardiac failure. In spite of hypervolemia, normal or a decrease in ductus venosus preload corresponds to effective ventricular contractility.

A reduction of PLI, PVIV, and PIV values in the fetuses of the high-output group indicates an increase in forward flow volume and decreased forward flow resistance in the ductus venosus. This increase of venous return is consistent with an increase of cardiac output. Of interest, umbilical vein pulsations in this group did not represent cardiac decompensation as seen in the low-output group because they developed in spite of low preload index or low central venous pressure. These pulsations signify the opening or dilatation of sphincter-like ductus venosus in benign response to hypervolemia instead of back pressure from cardiac decompensation.

We postulate that during the early stage of anemia, hydropic change can occur, the fetus effectively compensates by increasing the cardiac dimension and contractility, leading to an increase in S wave without increased central pressure. Congestive heart failure and increased atrial pressure do not develop in most fetuses with high-output hydrops fetalis if they are not in the end-stage. Additionally, a change in portocaval pressure gradient, which determines the ductus venosus blood velocity,[20] may play a role in increased a wave (lower PLI).

This study suggests that the normal fetal heart is extremely high, capable of compensation to cope with anemic hypoxia. Marked cardiomegaly because of anemia probably reflect high competency of adaptation, rather than cardiac decompensation. This is consistent with our observation that several cases of hydrops fetalis because of hemoglobin Bart's disease can survive in utero with reactive nonstress test in spite of marked cardiomegaly without other signs of increased central pressure.

Our findings imply that, in fetal anemia, local and systemic compensatory mechanisms are highly effective and may help the fetus to survive. On the other hand, some of these mechanisms at the same time increase the imbalance of Starling forces, resulting in enhanced interstitial fluid accumulation before the compensatory mechanisms break down.

Although anemia in this series was lethal and the Doppler studies in these fetuses are not of value, it could be a study model for less severe causes of anemia such as Rh isoimmunization or parvovirus B19. Although fetuses with high-output hydrops fetalis may have poor prognosis in overall, a better prognosis can still be expected, as long as the venous Doppler velocities are normal. Concerning hydrops fetalis because of anemia secondary to treatable causes, venous Doppler pattern may theoretically be used to differentiate hydropic fetuses with congestive heart failure or myocardium compromise, from one without heart failure. Thus, venous Doppler assessment may be helpful in predicting the prognosis and therefore in making a choice of management.

In conclusion, new insight gained from this study is that low-output and high-output hydrops fetalis have different abnormal venous Doppler waveforms. This study provides strong evidence that, in the fetuses with low cardiac output, myocardial dysfunction and cardiac decompensation is a primary cause of hydrops fetalis, whereas hypervolemia is the primary cause in the

TABLE 3
Comparison of the presence of umbilical vein pulsations among the both groups of hydrops fetalis

		Umbilical vein pulsations		
Group	n	Absent	Present	*P* value
High-output hydrops fetalis	50	36 (72.0%)	14 (28.0%)	< .001
Low-output hydrops fetalis	19	4 (21.1%)	15 (78.9%)	< .001

Tongsong. Venous Doppler in hydrops fetalis. Am J Obstet Gynecol 2010.

fetuses with high cardiac output and cardiac failure is a consequence only when cardiac compensatory mechanism is exhausted. Additionally, umbilical vein pulsations with normal or low preload index probably represent ductal opening in benign response to hypervolemia rather than myocardial dysfunction as seen in low cardiac output in fetal cardiac defects or in fetal growth restriction-related hypoxia. ■

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MATERNO-FETAL MEDICINE

Reference range of fetal splenic circumference from 14 to 40 weeks of gestation

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Abstract
Objective To create a nomogram for fetal splenic circumference of normal fetuses.
Materials and methods A prospective, cross-sectional study was undertaken on normal pregnancies with certain date from 14 to 40 weeks of gestation. All fetuses were measured for fetal splenic circumference by tracing technique on transverse view of the fetal abdomen, using high-resolution real-time ultrasound with a 2–4 MHz convex transducer.
Results A total of 684 normal pregnant women between 14 and 40 weeks of gestation were recruited. Fifty-eight were excluded because of poor image quality and fetal abnormality. The remaining 626 were available for analysis. Quadratic equation model was best fitted to estimate the 5th, 50th and 95th percentile range of splenic circumference at each gestational week. Fetal splenic circumference was gradually increased with gestational age with fitted equation as follows: splenic circumference (cm) $= -4.181 + 0.456$ (GA) -0.001 (GA)^2 ($r = 0.942$, $p < 0.001$). The table of nomogram for various percentile ranges was constructed.
Conclusion A normal reference range of fetal splenic circumference for each week of gestational age during 14–40 weeks is established.

Keywords Fetus · Splenic circumference · Nomogram · Ultrasound

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Introduction

The spleen is visualized on a transverse plane as a solid organ posterior to the stomach. It is hematopoietically active, especially during 12–24 weeks of gestation. Fetal anemia as well as intrauterine infection can lead to extramedullary hematopoiesis, resulting in an increase in splenic size. Splenic size measurement may be helpful in the prediction of fetal anemia [1]. There is a significant correlation between splenic circumference and severe fetal anemia [1, 2]. For example, Bahado-Singh et al. [2] found that splenic circumference was an excellent predictor of severe anemia in Rh-alloimmunized nonhydropic singleton fetuses (sensitivity 100%, and specificity 94.7%). Furthermore, splenomegaly may also be associated with other causes such as non-immune hydrops fetalis, infection, and inborn errors, e.g. Gaucher disease and Nieman-Pick disease. Therefore, a nomogram can be useful in detecting abnormalities of the fetal spleen and thus provide a new complementary method to identify possible fetal disorders [3]. Consequently, normative data of splenic circumference have been published by several authors. Nevertheless, most are based on small sample size and most do not include fetuses in early second trimester, which is particularly helpful in the prediction of severe anemia occurring in early gestation such as Hb Bart's disease [4, 5]. Additionally, some did not provide table of predictive values for each gestational week, but only summary of raw data of each gestational week which are difficult for clinical use [6]. Importantly, as already known, outline border of the spleen is often unclear and is not easily discriminated from surrounding structures by ultrasound machines of earlier models, used in most previous studies. Moreover, several reports suggest that the average birth weight of the Eastern fetuses is lower than that of the Western and a

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different standard of ultrasound-based fetal growth is needed for different population [7, 8]. Therefore, it is very important to create a new nomogram of the fetal splenic circumference that can be used more appropriately for its own population.

The objective of the present study was to establish a nomogram of fetal splenic circumference from 14 to 40 weeks of gestation based on high-resolution machine and adequate sample size, in the hope that this may be baseline normative data in evaluation of fetuses at risk of fetal anemia.

Materials and methods

This prospective cross-sectional descriptive study was undertaken between 1 September 2007 and 31 October 2009 at Maharaj Nakorn Chiang Mai Hospital, Chiang Mai University, Thailand with an approval of the research ethical committee. Pregnant women attending our antenatal care clinic were recruited into the study with written informed consents. Inclusion criteria included (1) accurate gestational age based on sonographic dating in the first half of pregnancies and reliable last menstrual period, (2) gestational age between 14 and 40 weeks and (3) low-risk pregnancy without known obstetric and medical complications. Exclusion criteria included (1) fetal malformation or chromosome abnormalities, (2) small- or large-for-gestational age (less than 10th or more than 90th percentile for gestational age), (3) multifetal pregnancy, and (4) poor imaging of fetal splenic border outline on ultrasound examination. All sonographic measurements were performed by experienced sonographers with high-resolution real-time machine, Voluson E8 (GE Healthcare, USA) equipped with transabdominal 2–4 MHz curvilinear transducers.

In each examination, a routine standard sonographic examination, including fetal anomaly screening and fetal biometry was first performed. On splenic measurement, the ultrasound transducer was oriented to obtain the transverse view of the fetal upper abdomen and then the fetal spleen is identified as a triangular or crescent-shaped structure with homogeneous echo density, nearly or slightly echogenic than the liver, posterior to the fluid-filled stomach and lateral to the left adrenal gland, as shown in Fig. 1. The left adrenal is occasionally imaged between the spine medially and the spleen laterally. Splenic length and width were measured and splenic circumference was measured by the trace method and by calculation from the formula: circumference = (length + transverse dimension) × 1.57. The three best measurements were averaged and digitally recorded in the computerized record form. Baseline demographic and obstetric data of the pregnant women were also recorded digitally at the same time. The pregnant

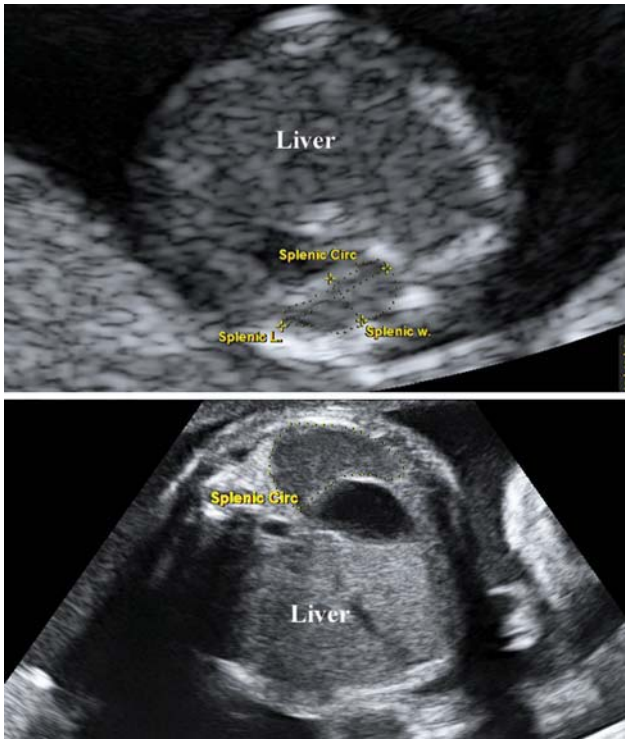

Fig. 1 Measurement of the fetal spleen at 14 weeks of gestation in position of left side down (*upper*) and the fetus at 32 weeks of gestation in position of left side up (*lower*)

women were taken care by standard antenatal care. The stored data were analyzed for the 5th, 50th and 95th percentile of fetal splenic circumference for each week of gestation. Regression analysis was used to express the correlation between the splenic circumference and gestational age. Predicted percentile curves for the splenic circumference values were derived using the statistical package for the social sciences (SPSS) version 17.0 (Chicago, USA).

Results

A total of 684 normal pregnant women between 14 and 40 weeks of gestation were recruited. Fifty-eight were excluded because of poor image quality especially in cases of early gestation and fetal abnormalities; birth weight of less than 10th percentile, macrosomia, and fetal structural or chromosome anomalies, including congenital heart defects, abdominal wall defect, and diaphragmatic hernia. The remaining 626 measurements were available for analysis and the average measurement per week was 20.2, ranging from 11 to 30 per week. The mean (±SD) maternal age was 27.5 ± 5.4 years (15–43 years). Two-hundred and eighty-six (45.69%) were nulliparous. A comparison between splenic circumference derived by calculation technique and

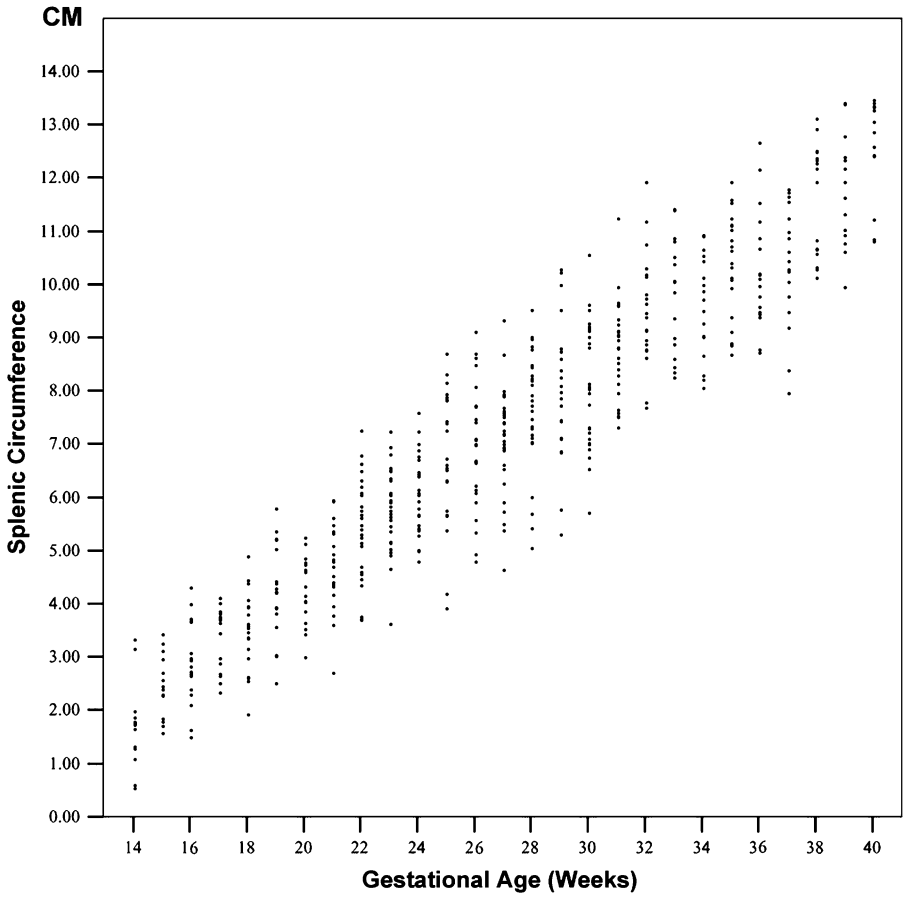

Fig. 2 Scattergrams of splenic circumference for each week of gestation, the *three lines* represent predicted value of median and 95% confidence interval

tracing technique shows that the values by tracing technique are significantly higher than that derived from the formula (paried-*t* test, two-tailed <0.001, Mean difference 15.4% with standard deviation 8.3%). Splenic circumference (tracing technique) was found to increase as a quadratic equation throughout the second and third trimesters as shown in Fig. 2. The distribution of splenic circumference shows normal in all gestational weeks, using Kolomogorov–Smirnoff test. Regression analysis was used to identify the best regression model for their relations. The best fitted equation is quadratic function as follows: splenic circumference (cm) $= -4.181 + 0.456(\mathrm{GA}) - 0.001(\mathrm{GA})^2$ ($r = 0.942$, $p < 0.001$). The table of predicted reference range for 5th, 50th, and 95th percentile of splenic circumference was constructed (Table 1). The results show a gradual increase in splenic circumference over the period of 14–40 weeks as shown in Fig. 2. The interobserver mean difference for the measurement of splenic circumference was 5.4%.

Discussion

The nomogram of splenic circumference or splenic size may be served as a basis for early detection of hydrops fetalis or fetal anemia. Additionally, ultrasonographic identification and measurement of the fetal spleen in utero is a useful indicator of fetal spleen growth and should facilitate detection of in utero splenomegaly, asplenia, and other abnormalities. Nevertheless, the problems of available nomograms in the literature are based on small sample size (e.g. only 2–6/week), not including early gestation, machine in the era of low resolution, and, most importantly, the splenic circumference values derived by calculation from length and width which did not exactly represent spelnic parameter, likely to be less accurate than tracing the splenic perimeter. This study showed that the two methods of measurement were significantly different. Theoretically, tracing method is more reliable than that derived from the length and width since the spleen shape is triangular or crescent shape rather than simply geometric. Therefore, we use tracing method for nomogram construction and we suggest not to use splenic circumference values derived from width and length. This study has shown that the fetal splenic circumference in normal Thai fetuses gradually increases with quadratic growth pattern unlike linear growth as reported in other studies. This may be due to very small sample size in most previous studies [2, 3, 9, 10] to express a little decelerating splenic growth in late pregnancy.

Table 1 Nomogram of predicted splenic circumference values (cm) for each gestational week at 5th, 50th, and 95th percentile

Gestational weeks	Splenic circumference (cm)		
	5th percentile	50th percentile	95th percentile
14	0.01	1.96	3.91
15	0.43	2.38	4.33
16	0.85	2.80	4.74
17	1.27	3.21	5.15
18	1.68	3.62	5.56
19	2.09	4.03	5.97
20	2.50	4.44	6.38
21	2.91	4.84	6.78
22	3.31	5.25	7.18
23	3.71	5.65	7.58
24	4.10	6.04	7.98
25	4.50	6.44	8.38
26	4.89	6.83	8.77
27	5.28	7.22	9.16
28	5.67	7.61	9.54
29	6.05	7.99	9.93
30	6.43	8.37	10.31
31	6.81	8.75	10.69
32	7.19	9.13	11.07
33	7.56	9.50	11.44
34	7.93	9.87	11.81
35	8.30	10.24	12.18
36	8.67	10.61	12.55
37	9.03	10.97	12.91
38	9.39	11.33	13.28
39	9.75	11.69	13.64
40	10.10	12.05	14.00

Though the normal reference ranges in this study are comparable with those in previous reports [2, 3, 9, 10], probably it could not be compared due to the fact that we use tracing method which is proved in this study to give higher values. In fact, the normal reference values may be somewhat lower due to racial factors. Therefore, we suggest the need to construct separate nomogram for each specific population. Of pregnancies in the Eastern, the nomogram from this study may be more appropriate to apply than in those of the Western. Although our data seem to be comparable with those reported by Chawanpaiboon et al. [6], it is hard to compare, since their nomogram is based on raw data, not predicted values derived from the fitted equation, and the values swing from week to week which is difficult for clinical application.

Oepkes et al. [1] found a significant positive correlation between spleen perimeter and fetal hemoglobin deficit and they concluded that fetal spleen measurements may be a useful adjunct to ultrasonographic evaluation in the management of severe alloimmunized pregnancies. Likewise, Leung et al. [11] found that fetal splenic size assessment may be helpful in predicting anemia in fetuses with Hb Bart's disease. Anemia in hemoglobin Bart's disease usually occurs late first trimester or early second trimester [4, 5]. Therefore, the nomogram including fetuses in early gestation, like in this study, may probably be more useful.

The strength of this study include that (1) large sample size for each gestational week, (2) the splenic circumference values derived from tracing technique, (3) examination with high-resolution ultrasound machine, and (4) the nomogram including fetuses in early second trimester. This may particularly be helpful in predicting severe anemia occurring in early gestation such as Hb Bart's disease. Moreover, inter-observer variations of the measurement are well acceptable.

One limitation of this study is that, though this measurement is simple, in several fetuses in position of left side up and the spleen is in area of rib acoustic shadow, border outline of the spleen could not be clearly visualized, leading to excluding from the study, suggesting inability to apply to all fetuses in real practice.

We believe that this nomogram may be helpful in clinical practice such as evaluation of fetal intrauterine infection or fetal anemia, especially in isoimmunized pregnancy [2] or in pregnancy with a hemoglobin Bart's fetus [12]. Recently, the splenic volume has also been studied [13], however, it is difficult or not practical in actual use. We believe that simple measurement of fetal splenic circumference is more practical and can better represent fetal size in actual practice due to its higher reproducibility, as indicated by low inter-observer variations in this report.

In conclusion, a nomogram of fetal splenic circumference from 14 to 40 weeks is established. This may be helpful in the assessment for some fetal conditions associated with spleen size, especially when fetal anemia suspected. However, the effectiveness of this tool remains to be tested by further studies.

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Conflict of interest statement None.

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Table 1. Two-by-two table shows the diagnostic indices of simple OF in predicting alpha-thalassemia-1 and beta-thalassemia trait among nonanemic pregnant women (HbE trait is excluded)

Simple OF test	Carrier status		Total
	normal	carrier	
Negative	264	0	264
Positive	99	54	153
Total	363	54	417

Sensitivity: 100% (54/54); specificity: 72.7% (264/363); positive predictive accuracy: 35.3% (54/153); negative predictive accuracy: 100% (264/264).

Table 2. Two-by-two table shows the diagnostic indices of simple OF in predicting alpha-thalassemia-1 and beta-thalassemia trait among nonanemic pregnant women (HbE trait is included)

Simple OF test	Carrier status		Total
	normal	carrier	
Negative	274	0	274
Positive	149	54	203
Total	423	54	477

Sensitivity: 100% (54/54); specificity: 64.8% (274/423); positive predictive accuracy: 26.6% (54/203); negative predictive accuracy: 100% (274/274).

HbA2 levels of 4.1–9%, carriers for beta-thalassemia and positive test for PCR (SEA type), carriers for alpha-thalassemia-1 were found in 28 (6.7%) and 33 (7.9%), respectively. Sixty women had Hb E trait and were excluded. Fifty-four of the remaining 417 women had an abnormal or positive gold standard test (7 of them were positive for both alpha-thalassemia-1 and beta-thalassemia carrier).

The outcomes of pregnancies could be summarized as follows: The average gestational age of all recruited women was 38.3 ± 4.8 weeks (±SD). Most newborns (70%) had birth weight between 2,500 and 3,500 g. Most of the patients had vaginal delivery and cesarean section rate was 20%.

Among the total of 54 carriers (alpha-thalassemia-1 or beta-thalassemia, or both), all had a positive test of simple OF screening test, giving a sensitivity of 100.0% and a specificity of 72.7%. The diagnostic indices (sensitivity, specificity, positive predictive value, and negative predictive value) are presented in table 1.

Of 60 women with Hb E trait, 10 (16.7%) had negative simple OF and 50 (83.3%) had positive simple OF. If Hb E trait were included, the specificity and positive predictive values were decreased to 64.8 and 26.6%, respectively, as presented in table 2.

Discussion

The incidence of severe thalassemia (homozygous beta-thalassemia, Hb Bart's hydrops fetalis and beta-thalassemia/HbE disease) is very common in Southeast Asia and they need to be controlled, especially by a prenatal control strategy [2, 12, 13]. However, every method of prenatal control needs effective screening and screening methods should be highly effective, simple, cheap, and less time-consuming. MCV or standard OF is the most commonly used for screening for both beta-thalassemia trait and alpha-thalassemia-1 trait (one test for both carriers). MCV is convenient and has acceptable accuracy when it is determined with an automated cell analyzer, but the machine is rarely available in rural areas. Standard OF (0.32–0.36% saline solution) is one of the most commonly used screening tests for such a purpose in the prevalent areas, though the techniques of the test are widely different. The accuracy of the test in real practice is varied but it has rather high sensitivity and acceptable specificity. However, the best technique still needs to be sought for [2, 14, 15]. Our extensive experience at Maharaj Nakorn Chiang Mai Hospital with standard quantitative OF (0.45% glycerin saline solution) has shown high sensitivity in detecting beta-thalassemia and alpha-thalassemia-1 trait [2, 10]. However, several rural areas with high prevalence of the disease have no spectrophotometer required for the standard OF. Therefore, simple OF has been developed to overcome that problem, and now simple OF is widely used in Southeast Asia. However, its accuracy has never been tested to determine whether it has a comparable prediction as standard OF or not. This study shows that simple OF has as high accuracy as that of standard OF reported in previous study [8]. Based on our findings, simple OF has high sensitivity of 100%. This implies that a carrier of severe thalassemia (alpha-thalassemia-1 or beta-thalassemia) could not be missed with the use of simple OF as a screening test because all cases of the carriers will have a positive simple

OF. This is the greatest advantage of the simple OF, permitting us to recruit all of the couples at risk to undergo prenatal diagnosis.

Like the standard or conventional OF test, the simple OF, however, is not perfect. The specificity of simple OF in this study is not so high and the false positivity is rather high (nearly 30%). This means that there are a significant number (about one-third) of pregnant women who would unnecessarily be further tested with more expensive tests (HbA2 levels and PCR) to confirm the diagnosis. However, these expensive tests can be reduced if both of the couples are screened. The definite tests for carriers are necessary only when both of them have a positive screening test. If only one of the couple has a negative screening test, both of them are not at risk of having a child with severe thalassemia. With this approach, the gold standard test may be needed in only 10% of the screened population. Overall, due to its high sensitivity with acceptable specificity, very low cost, simplicity and rapidity of testing and availability, this test should be encouraged for the continuation of wide use.

Simple OF, like other OF techniques or MCV, does not effectively screen the HbE trait. We have found that more than 10% of pregnant women with the HbE trait had normal simple OF. Therefore, this is another limitation that may be encountered in the use of simple OF as a screening test, and other screening tests should be added for HbE screening if beta-thalassemia/HbE disease is also intended to be controlled.

In conclusion, simple OF is a good method for screening the alpha-thalassemia-1 and beta-thalassemia traits in pregnant women. It has high sensitivity and acceptable specificity. Its accuracy is, at least, comparable with standard OF. Because of its high efficacy, along with its other known advantages including simplicity, rapidity in testing and very low cost, simple OF may be considered for routine screening thalassemia carriers in highly prevalent areas.

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